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CLINICAL AND EXPERIMENTAL

PHYSIOLOGY, CORRELATIONS AND TECHNIC OF THE VAN DEN BERGH REACTION ICTERUS INDEX AND QUANTITATIVE SERUM BILIRUBIN*

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MANN and Rich have shown that if the liver of a dog is extirpated intense tissue jaundice rapidly develops. Mann found that the beginning of this icterus may be detected in the serum of the animal as early as fifteen minutes following hepatectomy. Thus the extrahepatic origin of bilirubin in a mammal is demonstrated, as well as the rate at which it is produced. Mann also observed that the serum bilirubin obstructed by the removal of the excretory organ was direct negative in terms of the van den Bergh reaction. He further found that if prior to hepatectomy, the common duct had been ligated long enough to cause a jaundice in which the serum contained direct positive bilirubin, the icterus again rapidly intensified after hepatectomy, but this was again due to the accumulation of direct negative bilirubin, since the level of the direct positive pigment remained unchanged following the removal of the liver. The causative factor of the jaundice in each of these experiments is obviously obstruction; the difference in the nature of the serum bilirubin being dependent on whether the site of the obstruction be placed above or below the liver parenchyma where it has long been recognized that direct negative bilirubin is changed to the direct positive form in the mammalian organism.

The excretion of bilirubin by the liver is in many ways analogous to the excretion of the nonprotein metabolites by the kidneys. In either case the excreted substances are produced in another part of the body. When uremia exists, a disturbance in the excretory organ is postulated and not an over-

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production of the metabolites. Bilateral nephrectomy would not be acceptable proof that the kidneys could have nothing to do with anemia. The balance between production and elimination is regulated chiefly by the efficiency of the excretory organ.

Van den Bergh states "When more bilirubin is brought to the liver cells more is excreted, but also more is retained by the blood. . . . if the liver cells are compelled to reject some of the excess, more may also appear in the bile. . . . this is characteristic of acholic icterus. . . . it would not be correct to call this type of icterus nonhepatic. . . . the term 'hematogenous' icterus is incorrect since every icterus is, in a sense, hematogenous, although bilirubin is never formed in the blood stream. . . . this form of icterus is due to insufficient capacity of the liver cells to excrete bilirubin be it relative, following greater influx or be it absolute due to functional liver disturbance. . . . its best name is dynamic or *functional* icterus." Thus a form of icterus commonly known as "hemolytic" is seen in the light of functional obstruction.

Von Bergmann estimates that in the average normal 70 kilo human adult the daily bilirubin production is about 500 mg. which in terms of 100 cc. units of blood serum volume may be computed as approximately 14.3 mg. The normal bilirubin level in human serum does not exceed 0.2 mg. per 100 cc., except for occasional elevations to about 0.35 mg. Visible jaundice may appear in the white race when this level rises to 1.5 or 2.0 mg. and may often be detectable in the sclerae at much lower levels. The excretion rate must be efficiently maintained to preserve the balance and prevent a constantly ascending icterus, whether the excretion threshold be normal or pathologically elevated. The elimination of bilirubin is further complicated by the evidence that during excretion it is changed from the direct negative form to the direct positive form, thus introducing a conversion mechanism as an integral part of the excretion process.

The difference between these two forms of bilirubin has been investigated by several workers among them Blankenhorn, M'Gowan and Collinson and Fowweather. Blankenhorn found that there was present in bile and in many icteric sera a form of bilirubin which would pass through an animal membrane showing that since it acted as a diffusate on dialysis it must be in the crystalloid state. He found however that this was a relatively unstable form for on repeated dialysis of the diffusates, less and less would pass through the membrane, indicating that it readily reverted to a nondialyzable, or colloidal, form. Collinson and Fowweather applied this observation to the van den Bergh reaction and found that the crystalloid form alone gave the direct positive reaction while the nondialyzable or colloidal form was direct negative, reacting with the diazonium salt only after the addition of alcohol and giving the "indirect" or quantitative reaction. M'Gowan found that the use of buffer solutions and a change in hydrogen-ion concentration toward greater alkalinity could cause a direct negative reaction to become direct positive. It is rational to conclude then that the difference between the

two forms of bilirubin is largely a matter of physical state the direct negative being free bilirubin a suspensoid colloid the direct positive being a true crystalloid

Sufficient data are available to imply that nascent bilirubin as it is produced extrahepatically in a mammal is in the colloidal state and that it is converted by the liver parenchyma during excretion to an ionizable form which is a true but unstable crystalloid. This latter form has been described by different workers as an ammonium salt of bilirubin and as sodium hydrogen bilirubinate. That the liver parenchyma is ordinarily impermeable to colloids was found during the development of the Graham test providing further evidence of the necessity for a physiologic conversion mechanism as an integral part of the excretion process for bilirubin.

The experiments of Naunyn and Minkowski, expanded by McNee have shown that in birds the Kupffer cells play the most important rôle in the formation of bilirubin from hemoglobin while in contrast with mammalian physiology, the bone marrow and spleen are relatively unimportant in this respect. Since bird serum normally contains very little bilirubin the pigment must be passed on by the Kupffer cells across the subendothelial lymphatics of the liver lobules to the polygonal cells as rapidly as it is formed. Hence in birds a dual rôle of these cells in formation and excretion is implied. In mammals however it is clear from the work of Mann that the rôle of the Kupffer cells in formation of bilirubin is quite insignificant, and then only possible remaining function if any would be largely excretory, as the acceptors of nascent colloidal bilirubin to convey the pigment across the lymphatic spaces from the sinusoidal stream to the polygonal cells for conversion and excretion. As actively phagocytic sessile cells they are known to have an affinity for colloidal particles although bilirubin appears to be the only colloidal substance for which the subendothelial lymphatic spaces are bridged. If such a function be granted then their relative permeability to nascent bilirubin would determine the excretion threshold.

The direct delayed van den Bergh reaction can rationally be interpreted as indicative of the presence of bilirubin undergoing the transition from the colloidal to the crystalloid state manifesting by the length of the delay period its proximity to one or the other of the two extremes. Colloidal particles breaking down into polymerized, then simple, crystalloid molecules would be expected to react in an aqueous medium with a speed characteristic of their relative solubility. Many similar chemical reactions exhibit definite delay periods as for example the colloidal gold reaction with spinal fluid. The source of direct delayed bilirubin would again be liver parenchyma since it is there that the conversion takes place.

Should an obstructive process exist in or below the liver parenchyma complete enough to produce intense pigmentary congestion in the excreting cells much converted pigment would escape into the blood stream by way of the subendothelial lymphatic spaces of the liver lobules but also free bilirubin normally conveyed to the sinusoids at a rapid rate would be denied entry to the polygonal cells resulting in an icteric serum containing both forms of bilirubin. Such sera would obviously give direct positive reactions the direct negative

content being determinable by deducting from the quantitative total the quantitative determination on the direct positive reaction alone. Only a trace of crystalloid bilirubin suffices to give rise to a direct positive reaction, for they may frequently be encountered in sera with normal icterus indices and bilirubin content in lobal pneumonia, septicemia, multiple liver abscesses, cardiac failure, secondary syphilis, metastatic carcinoma of the liver, and following accidental or operative trauma.

The icterus index may be defined as a physical measurement of the yellow color intensity of serum, not directly proportional to the quantitative bilirubin content. Since bilirubin may occur in serum as a crystalloid, in true solution, or as a suspensoid colloid subject to variations in the size of the particles suspended, many of the well-known discrepancies between the icterus index and the total bilirubin content may be understood. A color producing substance in true solution might be expected to impart a greater color intensity to its solvent than an equal amount occurring in the medium as a suspended colloid. Hence in a serum with a given icterus index much less crystalloid pigment need be present to cause that color intensity than would be required if it were in the colloidal state, therefore, it may be inferred that a serum yielding a relatively high bilirubin content contains a large part of the pigment in the colloidal state, and conversely, if the bilirubin content is relatively low, most of it is present in true solution. The icterus index, then, may be regarded as a function as well of the physical state of bilirubin as of its total quantity.

CORRELATION OF THE THREE TESTS

Hubbard has attempted to correlate the icterus index with the length of the delay period of the van den Bergh reaction, but found no definite point of demarcation in a large clinical series, except that as the index rose, the percentage of direct positive reactions increased and the shorter delay periods became more evident. Woodruff and his coworkers made some very interesting findings in a correlation of the icterus index and the quantitative bilirubin. To small amounts of normal dog serum they added known quantities of pure bilirubin, rendered soluble by alkali, and then, deducting the icterus index of the normal serum, determined the icterus index caused by the added bilirubin. On a graph with the known quantities of bilirubin as ordinates, the corresponding icterus indices were plotted in as the abscissae. The bilirubin range was 0-10 mg per 100 c.c., and the icterus index range 0-100. Then "line of maximum density" bisected the graph diagonally from 0 to the icterus index 100 and the bilirubin 10, thus indicating that a quantitative bilirubin of 61 should give an index of 61, a bilirubin of 29 an index of 29, a relation expressed by a shift of the decimal point. There is shown, however, a striking characteristic in the variation of the points from the line of maximum density. Below the icterus index of approximately 16 practically all of the bilirubin values run above the line, between 16 and 30 there is evidence of a definite downward loop below the line, beyond 30 they vary widely on both sides of the line. It is this downward loop which commands attention, because it may be found clinically in exactly the same zone in lobal pneumonia in the white race, in pernicious anemia, familial jaundice and in the newborn (placenta

blood) It has not as yet been observed in the black race in any of these entities, for it definitely does not occur in the ascent of the icterus in lobar pneumonia in the negro, and pernicious anemia and familial jaundice are so rare in the black race that none have as yet been studied

The accompanying charts present the results of a correlation based on approximately 1700 sera examined by the three test method, from over 700 patients representing a wide variety of clinical entities Since these tests were done by a single observer using a uniform technique, differences may be regarded as fairly valid in significance, with the factor of error a constant

Chart 1 demonstrates the possible variation in total bilirubin content of a serum having an icterus index from the normal zone to 60 It is obvious that the icterus index fails to conform consistently with any constant total bilirubin content ratio

There are no known cases of carotinemia in this group The relatively high bilirubin values in and near the normal zone of the icterus index were found in sera from patients with familial jaundice and pernicious anemia, in placenta blood, and in sera from patients suffering only from trauma, accidental (contusions fractures, deep lacerations bullet wounds), or operative In this traumatic group latent jaundice was a constant feature often manifest for a short time in the sclerae, and evidently produced by the formation and release of bilirubin from hemoglobin in the interstitial blood extravasation at the site of the trauma Direct positive van den Bergh reactions were very frequently encountered during the course of this icterus caused by trauma The relatively low bilirubin values in the higher zones of the icterus index were found in serial studies as any jaundice was subsiding, occasionally in cirrhoses, and in negroes If a line be drawn connecting the maximum bilirubin values in areas of high dot-density, two downward loops will be seen, a small one following the icterus index 10 and a very definite one between the index 16.6 and 30 These icterus indices, 10 and 16.6, have been found to be of extraordinary significance in a study of 224 cases of lobar pneumonia

Several points are demonstrated on Chart 2 (1) For a given icterus index the bilirubin content is higher when the pigment is direct negative (or long delayed), and in the colloidal state, than when the pigment is direct positive and a crystalloid (2) In sera with a direct negative (or long delayed) reaction a sudden increase in bilirubin content takes place when the icterus index arrives at 16.6 (3) As the icterus index passes 16.6, sera uniformly exhibited either a true direct positive reaction or an immediate golden accentuation on the addition of the diazo reagent This icterus index seems to be the upper limit of color intensity which can be produced by colloidal bilirubin (serum hematoidin) before a change takes place in the van den Bergh reaction

The data for the "dot-dash" line of direct negative bilirubin is derived from 200 sera from patients exhibiting icterus due to trauma exclusive of those sera in which zonal positive or direct positive reactions were present, hence the use of the term "blood extravasations" in the legend

On Chart 3 are plotted the average bilirubin contents for given icterus indices found in pernicious anemia (15 cases), familial jaundice (12 cases),

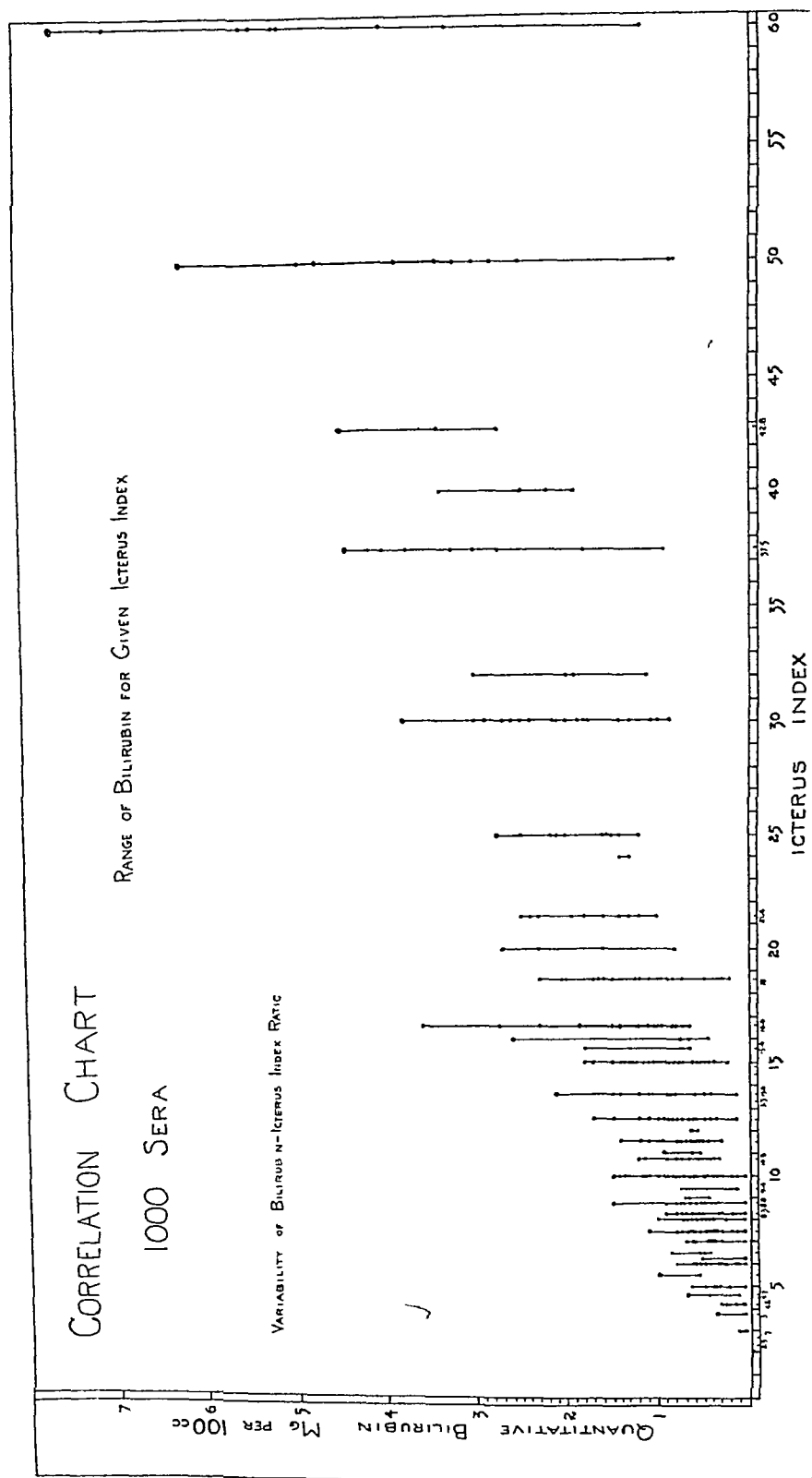
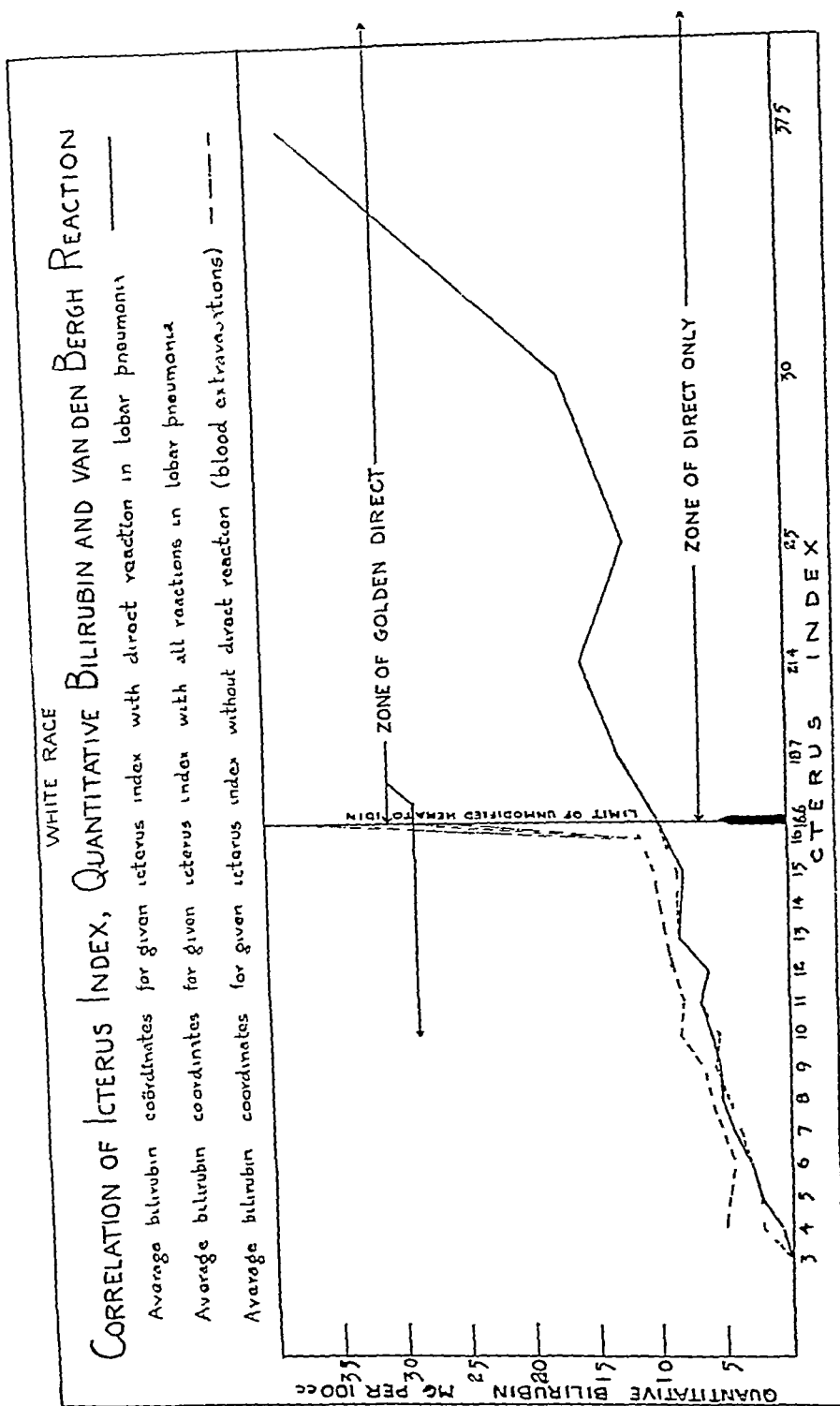


Chart 1



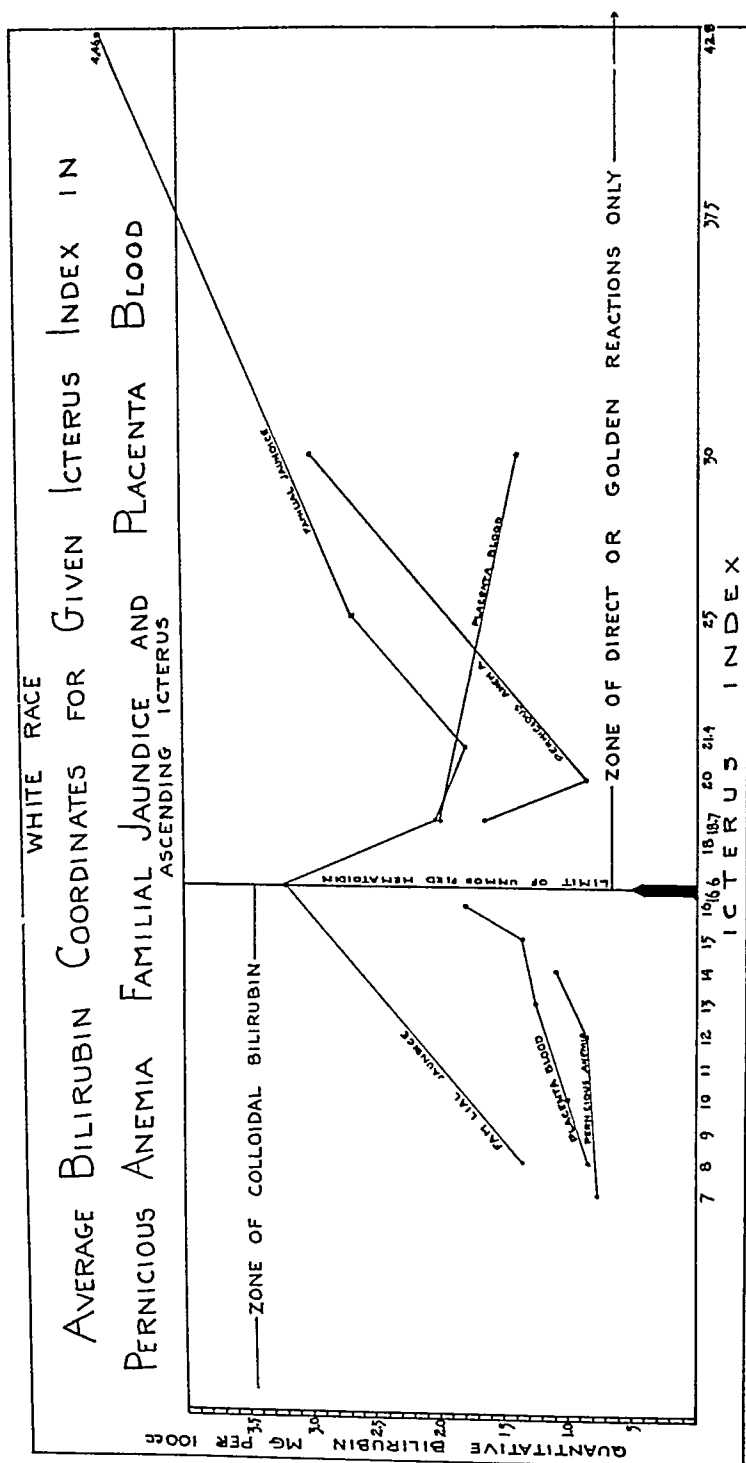


Chart 1

"HEMATOIDIN CRISIS" IN FAMILIAL JAUNDICE
HAYEM-WIDAL TYPE

Harry M Detroit Receiving Hospital 114270

The graph displays two primary data series: Bilirubin (mg per 100cc) and Index (Hayem-Widal type), plotted against the Day of Observation (1 to 19). A third series, Direct van den Berg delay period, is shown at the bottom of the graph.

Bilirubin Data (mg per 100cc):

Day of Observation	Bilirubin (mg per 100cc)
3	28
6	35
9	28
11	25
15	15
17	15
19	12

Index Data (Hayem-Widal type):

Day of Observation	Index
3	17
6	17
9	25
11	25
15	15
17	15
19	8

Direct van den Berg Delay Period (Scale 1 to 10):

Day of Observation	Direct van den Berg Delay Period
3	2
6	2
9	6
11	6
15	2
17	1
19	1

jaundice have been observed, one exhibiting a direct positive reaction throughout the course of the icteric exacerbation, and the other an exception to the previous findings, exhibiting icterus indices for five months in the 16.6 to 30 zone without evidence of either a downward loop or a golden reaction. The

*Greene has reported direct positive reactions in occasional cases of pernicious anemia and familial jaundice.

delay periods in the reactions, when not direct positive were found to be shorter in placenta blood than in pernicious anemia and longest in familial jaundice

Chart 4 exhibits serially "the middle of the icterus index 16.6" in a specific case of familial jaundice. The dotted line at the bottom of the chart where the van den Bergh reaction curve is plotted in terms of the length of the delay period indicates by the circles on the *direct* line that with the rise of the icterus index from 16.6 to 25 accompanied by the decrease in serum bilirubin the immediate golden reaction with the diazo reagent developed. The red direct reaction was consistently absent except for the development of an eight-minute delay period on the fifteenth day of observation as the accumulated bilirubin was passing through the liver parenchyma. The masking of direct negative bilirubin at the icterus index 16.6 without increasing the yellow intensity of the serum can be explained only by assuming the formation of larger colloidal aggregates of the pigment, unable to enter the liver parenchyma terminated by a spontaneous physical change by which a emulsalloid derivative was evolved to which the liver was permeable, producing a greater color intensity in serum, although quantitatively less, because of greater solubility as indicated by the immediate golden color change in the aqueous medium of the direct van den Bergh reaction.

TECHNIC OF TESTS

The following details of technic describe the uniform method used in this study. All three tests may quickly be done on each serum by the continuity of technic. They have been found adaptable only to a study of the changes in the icterus of a given clinical entity, and are usually of little or no value as in and in differential diagnosis when used separately or in single determinations.

Icterus Index.—Using a Duboseq colorimeter the serum from about 10 cc. of fresh blood is compared directly with a standard 1:10,000 solution of potassium dichromate, which arbitrarily represents unity. The standard is usually set at 15 mm. The serum must be clear and yellow, free from hemolysis visible to the eye, turbidity, or murky brownish color. The comparison is best made using natural morning light from a window. The calculation is as follows:

$$\frac{\text{Reading of standard}}{\text{Reading of serum}} = \text{icterus index}$$

The normal range is from 3 to 5 signifying that human serum has from 3 to 5 times the yellow color intensity of the arbitrary standard. There is no racial difference known. Latent jaundice may still be present even though the icterus index lies within normal limits, its detection then depending on the van den Bergh reaction and the quantitative bilirubin determination. Conversely, high icterus indices may not be indicative of abnormal bilirubin content when the serum color is other than clear and yellow. The possibility of an increase in lutens, lipochromes, and carotinoids must be considered, as well as, according to van den Bergh, hematin icterus.

Van den Bergh Reaction.—After determining the icterus index, 1 cc. of serum is poured from the colorimeter cup into an accurately graduated centrifuge tube. The tube is slanted and from a pipette 0.5 cc. of Ehrlich's diazo reagent (freshly mixed) is overlaid on the serum (technic originally used for urine). An immediate reddish, amber, or port wine color reaction at the contact zone is indicative of a positive zonal reaction. Serum and reagent are then shaken and mixed so that a small amount of serum at the lower tip of the tube remains unmixed and can be used as a control of the color change above. The tube should

be watched for at least ten minutes for evidence of the beginning of a reddish, amber, or port wine color change. If the color change occurs at once, it may be called direct positive. Should the reaction not begin until a few minutes have elapsed, the delay period should be stated as accurately as possible. When no change occurs up to ten minutes the reaction may, for practical purposes, be called direct negative. If at the zone of contact or on mixing a sudden golden accentuation takes place, it may be called direct golden positive. These readings should also be made in natural daylight, preferably with a glazed glass window as a background. Normal sera are usually direct negative in the ten minute interval.

Most of the prevailing confusion in the interpretation of this test arises from the use of the term "biphasic," and from the fact that oxalated plasma evidently reacts differently from serum. The former frequently gives an immediate blue violet color,* while the latter practically never does, giving initially the reddish color alone when a direct positive reaction occurs. Hall has recommended that the term "biphasic" be discarded as meaningless, Andrewes disregards the quality of the color, and van den Bergh in 1928 reiterates, "no direct bluish color must appear." Hence, since the 'direct prompt,' or blue violet, reaction does not exist when serum is used, and all direct positive reactions could be called 'biphasic,' there is no particular reason for retaining such a nomenclature.

The results of the van den Bergh reaction may be recorded as

Direct positive
Direct delayed----minutes
Direct negative (10 minutes)

Positive zonal reactions may frequently be found when, after mixing, there seems to be no definite change up to two or three minutes. The golden accentuation should be recorded only when it can be demonstrated to a skeptic. It is often followed by a delayed red reaction.

Quantitative Bilirubin Determination—After the determination of the van den Bergh reaction, add 2.5 cc of 95 per cent alcohol, then 1 cc of saturated ammonium sulphate, and place in the centrifuge for a few minutes. On removal from the centrifuge, three layers will be seen, an upper pink or ruby colored layer in which essentially all the bilirubin is contained in alcoholic solution as 170 bilirubin, a middle layer of compacted precipitated proteins, usually colorless, and a lower layer of ammonium sulphate solution. Determine from the graduations on the tube the volume in cc of the upper colored layer and pour off some of it into a colorimeter cup for comparison with van den Bergh's standard cobaltous sulphate solution, which represents the color intensity produced in a similar reaction by exactly 0.5 mg of bilirubin per 100 cc. For pale color reactions set the standard at 2 mm in the colorimeter, for deep ruby reactions at 5 or 10 mm. The calculation is as follows:

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times \frac{\text{Dilution factor}}{2} = \text{mg bilirubin per 100 cc of serum}$$

By the use of the dilution factor the three tests may be run in continuity with as little as 0.4 cc of serum to work with, increasing somewhat, however, the factor of error.

$$\text{Dilution factor equals } \frac{\text{volume in cc of colored upper layer}}{\text{volume in cc of serum used}}$$

This quantitative determination (indirect reaction) is occasionally unsatisfactory due to anomalous factors in various sera. The alcoholic layer may not be comparable with the standard because of a yellow or orange color instead of the pink. A few drops of oxalic acid usually change the yellow color back to the pink, but a resulting turbidity often spoils the accuracy of comparison. In spite of the frequent difficulties encountered due to initial turbidity (removable by heating, by adding 1 to 2 drops of ether or an additional cc of alcohol), and to the yellow anomaly practical approximations can be made after the routine observation of a number of normal and icteric sera. With sera obviously deeply jaundiced little difficulty is encountered. Normal sera usually give either no grossly visible pink color in this reaction, or are but faintly reactive.

*The direct positive reaction on fresh bile is often blue violet but is usually red.

SUMMARY

Mann has shown that nascent bilirubin is produced extra-hepatically at a very rapid rate in the dog. The production rate in the human organism may be estimated as equally rapid, equivalent to approximately 14.3 mg per 100 cc of serum volume in twenty-four hours. Mann found that such nascent bilirubin is direct negative in terms of the van den Bergh reaction. It has long been known that direct negative bilirubin is changed to the direct positive form by the liver parenchyma in mammals as it is excreted. The difference in these two forms of bilirubin has been found to depend on the physical state of the pigment: the direct negative form being free nascent bilirubin occurring as a suspensoid colloid, the direct positive form being an unstable crystalloid salt of bilirubin. Since the liver parenchyma is ordinarily impermeable to substances in the colloidal state, the conversion of free bilirubin to a crystalloid salt is a necessary element of the excretion process.

In consideration of the relatively massive normal daily production of bilirubin, it is obvious that the excretion of the pigment by the liver must at all times be efficiently maintained to preserve the balance and prevent a constantly ascending icterus. From the experiments of Naunyn, Minkowski, and McNee it appears plausible that the Kupffer cells in mammals act as the acceptors of nascent colloidal bilirubin as their function in the excretion of the pigment, their relative permeability to the pigment being the threshold determinant under normal conditions and in functional icterus.

The direct delayed van den Bergh reaction can be interpreted in terms of the length of its delay period as indicating intermediate stages in the transition of nascent bilirubin to the crystalloid form, arising from pigmentary congestion in the liver parenchyma, the speed of the reaction depending on the relative solubility of the conversion products. Minute quantities of fully converted crystalloid bilirubin give rise to direct positive reactions in serum, and when both forms exist together, the reaction is direct positive.

The icterus index may be better understood as a "yellow intensity" index and not directly proportional to the bilirubin content, because of the many possible differences in the physical state and solubility of the pigment present.

In correlations of the results from over 1700 sera examined by the three test method, it has been found that: (1) The icterus index fails to conform consistently with any constant proportion of total bilirubin. (2) For a given icterus index the bilirubin content of the serum is higher when it exists in the colloidal state than when it is crystalloid. (3) Colloidal bilirubin accumulates in the blood stream at the icterus index 16.6, but fails to impart a higher color intensity to the serum in which it is suspended until it undergoes a physical change, expressed by the development of an immediate type of van den Bergh reaction. (4) In sera from the newborn, pernicious anemia, and familial jaundice a downward loop in total bilirubin content occurs as the icterus index rises from 16.6 to 30, accompanied by the appearance of an anomalous immediate golden accentuation in the direct van den Bergh reaction. One exception to statements 3 and 4 is cited.

A uniform technic for conducting the three tests in continuity on small amounts of serum is outlined, in which the innovation of overlaying the diazo reagent on the serum prior to mixing is proposed as a more sensitive method to detect the presence of crystalloid bilirubin. The terminology of Dr. A. A. Hijmans van den Bergh in the interpretation of the results of the reaction, as well as in the classification of jaundice as obstructive or functional, has proved most adaptable to the understanding of the pathologic physiology of icterus in the light of recent experimental work.

The results as presented in this paper comprise a part of an intensive clinical study of jaundice based on the serial application of the three tests for serum bilirubin, in which to date over 1700 sera from more than 700 cases have been studied, representing probably most of the conditions in which latent or clinical jaundice exists. Much of this work was done in the Department of Pathology at the Detroit College of Medicine and Surgery, of which Professor James E. Davis is the head, during the years 1929 and 1930.

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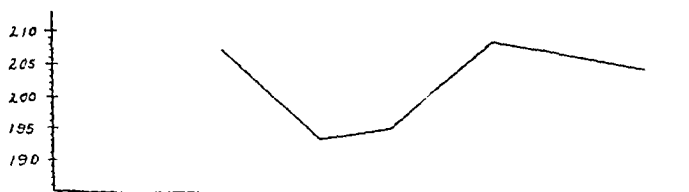
THE CATAMENIA AND OXYGEN CONSUMPTION*

By CHARLES L. WIBLE LINCOLN, NEB

ZUNTZ¹ in 1906 concluded that the intensity of oxidation processes in women changed very little during menstruation. Gephart and DuBois² (1916) confirmed these findings. Snell Ford and Rowntree³ (1920) found a rise just before and during the menses, with a marked fall after the period. Rowe and Eakin⁴ (1921) reported a rise of 13 to 18 per cent above normal previous to menstruation and a low rate during the period and for approximately two weeks after menstruation. Wiltshire (1921) and very shortly

Table I

Subject	No of menstrual cycles	Consumption of Oxygen (c c per minute)				
		1-4 days before menstruation	During menstruation		5-8 days after end of menstruation	2 weeks after end of menstruation
			1st	2nd		
G. W. II	1	210	189	200	209	206
	2	208	189	202	210	198
	3	201	192	180	197	199
	4	208	184	198	200	201
	5	211	200	209	213	210
	6	205	180	200	207	205
	7	211	203	202	205	208
	8	210	205	205	214	212
	9	208	199	179	207	204
	10	204	188	206	211	210
	11	195	195	190	200	201
	12	217	199	180	217	209
	Average	207	193	195	208	205



after Blunt and Dye⁶ found no elevation in the basal metabolism of women before and during the menses. Asher⁷ (1920) stated that "a direct influence of the sexual organs on metabolism does not exist." It is also stated by Geist and Goldberger⁸ that castration in women with previously functioning ovaries has no effect on basal metabolism. Wakeham⁹ (1923) concludes that there is a distinct fall in basal metabolic rate during or immediately after menstruation which is preceded by a rise. Benedict and Finn¹⁰ (1928) after extensive investigation of the problem reported that the oxygen consumption was lowest during the menstrual period and highest about one week after menstruation ceased. Griffith¹¹ (1929) found that menstruation lowered general metabolism.

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Women students are continually referred to our Student Health Dispensary for metabolism tests. Such conflicting reports as are above cited upon the metabolism of women during menstruation caused us to avoid the taking of such tests near the catamenia and also suggested a study of the problem. This study was initiated in September 1927.

Fortunate conditions for such a study exist in our Department of Physiology throughout the school year. The curriculum carries a course in Sex Hygiene for women students. The enrollment is always large. A number of these young women volunteered for the study and took considerable interest in our findings. In addition to this group several special students from our own department were available for the experiment over the entire period September, 1927 to September 1929.

TABLE II

SUBJECT	NO OF MEN- STRUAL CYCLES COVERED BY EXPERIMENT	AVERAGE CONSUMPTION OF OXYGEN (CC PER MINUTE)				
		1-4 DAYS BEFORE MENSTRUATION	DURING MENSTRUATION		5-8 DAYS AFTER END OF MEN- STRUATION	2 WEEKS AFTER END OF MEN- STRUATION
I M O	7	201	191	189	199	201
II G W	12	207	193	195	208	205
III H M	5	197	183	188	197	200
IV B B	5	185	181	175	183	187
V L H	8	195	195	188	198	197
VI B M	3	200	199	187	194	201
VII N A	5	196	186	190	193	197
VIII A C	5	189	187	183	195	193
IX A K	7	190	188	187	192	192
X M E	5	180	177	176	180	190
XI E F	5	188	182	181	196	186
XII B J	5	180	173	175	178	189
XIII M T	5	191	188	190	192	190
XIV O L	6	207	197	187	205	206
XV A P	4	198	196	191	199	201
XVI A O	3	187	187	189	196	197
XVII L C	5	197	188	186	200	199
XVIII C H	4	188	172	176	181	191
XIX L P	3	185	190	191	182	182
XX P M	4	186	177	178	189	193
XXI L K	8	206	198	201	205	206
XXII V H	4	196	186	185	198	199

The closed circuit oxygen consumption type of apparatus was utilized in this work. Routine records of buccal temperature, room temperature, pulse rate and barometric pressure were taken. All tests were made in the reclining position and in the early morning, fourteen to sixteen hours after the previous night's meal. The room temperature was maintained at a comfortable level with plenty of ventilation. Every subject was allowed a rest period of thirty minutes before beginning the test. Individuals with an indication of any circulatory deficiency were excluded from the experiment. Every step was taken to insure comfort for the subjects during the tests.

A test for leakage in the apparatus preceded every determination. To insure against any possibility of nervousness in connection with the first test of a series, each subject was allowed preliminary trials.

Tests were made on 22 different subjects covering a total of 118 menstrual cycles. Each cycle involved 5 tests distributed as follows: One to four days before the menstruation, two tests during menstruation, the first one usually falling on the first or second day, five to eight days after the cessation of menstruation and lastly two weeks after cessation of menstruation.

The figures shown in Table I are derived from subject No. 11 (G. W.). The curve accompanying Table I is the result of the plotting of averages. This curve represents in general the type of curve secured for each subject.

The average consumption of oxygen for each subject during each period of the experiment is given in Table II. It will be noted that with one exception, subject XIX, these results indicate a low oxygen consumption during menstruation. About one-half of the subjects showed one or two menstrual periods in which oxygen consumption during menstruation had a tendency to rise above the premenstrual consumption. These, however, were much in minority and the figures were in nearly every case lower than the consumption five to eight days after cessation of menstruation. The values secured do not indicate a premenstrual rise. The period of highest oxygen consumption seems to be two weeks after cessation of menstruation.

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STUDY ON A SERIES OF ARTHRITIC PATIENTS UNDER CONTINUOUS MONO-iodo-CINCHOPHEN TREATMENT WITH SPECIAL REFERENCE TO THE ACTION OF THE CINCHOPHEN MOLECULE ON THE LIVER TRACT*

By E P CORSON WHITL B A M D , PHILADELPHIA PA

SINCE 1923 when Wooster-Drought¹ described the first case of cinchophen toxicity a number of more or less carefully studied cases have been reported in the literature. These have been due not only to cinchophen itself but to its combinations and derivatives. The symptoms are always that of a moderate or severe toxic jaundice which often results in the death of the individual. The autopsy shows acute yellow atrophy of the liver with its associated pathology.

In the history of these cases it is impressive to note that the presence of symptoms of poisoning or the severity of the symptoms is not proportional to the length of time the drug has been taken or to the amount of the drug ingested. There are records of an almost daily consumption over periods of years, with no bad effects. This is further substantiated by the relatively small number of proved cases of yellow atrophy in relation to the amount of cinchophen and derivatives annually consumed in the United States (approximately 100,000 pounds per year).

The one patient, Case 22 responsible for this study has taken cinchophen almost continuously for six years. Poisoning also has occurred a week or weeks after discontinuing the administration. These facts indicate clearly that the responsible factor must be in the individual taking the drug.

Chemically, cinchophen is a phenylquinoline carboxylic acid. While the fate of these compounds in the system has been studied in the past, these investigations have thrown little light on the nature of the decomposition products and their elimination^{2, 3, 4}. However it is reasonable to believe that the liver plays a part in the decomposition of these complex products with the formation of secondary products of a more or less specific toxic action on this organ.

It therefore seemed to us to be of value, before instituting medication with cinchophen preparations, to examine the patients carefully for any evidence of liver or possible pancreatic dysfunction.

In this study a group of arthritis cases that under the previous treatment had failed to respond, were set aside for treatment with mono-iodo-cinchophen.

In all a history and physical examination were obtained with extreme care making especial effort to locate the original foci, so as to exclude any and all cases showing factors suggestive of disease or inefficiency of these or-

*From the Laboratories of the Orthopedic Hospital
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gans. The majority of the patients were drawn from the clinic service of Dr W J Taylor and the laboratory studies were made in the laboratories of the Orthopedic Hospital Philadelphia Pa

TABLE I

CASES	1	2	3	4	5
Diagnosis	Severe osteo arthritis deformans	Chronic infec tious	Chronic infec tious	Chronic hyper trophic	Arthritis deformans
Involvement	General	Hands, knees	Hands, knees, elbows	Knees	General
Age	63 years	58 years	54 years	62 years	32 years
Duration	9 years	22 years	3 years	4 years	3 years
Foci	Tonsils, teeth	Colon	Colon	Tonsils	Not found
Treatment period	17 weeks	19 weeks	19 weeks	19 weeks	17 weeks
W R	Neg	Neg	Neg	Neg	Neg
R B C *	3880000	4050000	4080000	4180000	4020000
	4610000	4420000	4100000	4080000	4080000
W B C	7800	6100	5800	6500	6700
	8000	7600	7800	6800	7000
Hb	64	79	78	83	79
	74	80	80	80	80
N P N	28.4	30.1	26.2	26.4	27.8
	28.2	30	26.1	27	28.7
Urea	13.4	15.1	12.8	13.2	13.9
	13.6	15.1	13.1	13.7	14
Uric Acid	2.1	2.8	2.7	2.3	2.7
	2.2	2.4	2.8	2.0	2.4
Creatinin	1.6	1.8	1.5	1.5	1.7
	1.6	1.6	1.7	1.9	1.3
Glucose	84	91.3	99.4	101.3	84.1
	91.1	88.7	101.2	98.6	84
Glucose tolerance	N	N	N	N	N
	N	N	N	N	N
Levulose tolerance	N	N	N	N	N
	N	N	N	N	N
Epinephrine response	Neg	Neg	Neg	Neg	Neg
	Neg	Neg	Neg	Neg	Neg
Icteric index	4.1	4.5	4	4	4
	3.9	4.4	3.7	4.1	4
Van den Bergh	D- ID-	D- ID-	D- ID-	D- ID-	D- ID-
	D- ID-	D- ID-	D- ID-	D- ID-	D- ID-
Rosenthal test	3% 15' 0 - 1 hr	4% 15' 0 - 1 hr	3% 15' 0 - 1 hr	4% 15' 0 - 1 hr	2% 15' 0 - 1 hr
	2.5% 15' 0 - 1 hr	3% 15' 0 - 1 hr	3% 15' 0 - 1 hr	5% 15' 0 - 1 hr	2% 15' 0 - 1 hr
Bile drainage	Neg	Not obtained	Not obtained	Neg	Not obtained
	Neg	Not obtained	Negative	Neg	Not obtained
Urobilogen	Neg	Neg	Neg	Neg	Neg
	Neg	Neg	Neg	Neg	Neg
Urine findings	Neg	Neg	Alb neg casts	Neg	Neg

*In each case the first line represents laboratory findings before treatment, the second line represents laboratory findings after treatment

N—Indicates Normal Neg—indicates Negative

Twenty one cases were accepted as being entirely free from any symptoms or signs of liver, duodenal or pancreatic disease as far as could be determined

The laboratory examination consisted in Wassermann tests, blood counts and differential blood chemistry, nonprotein nitrogen, uric acid, creatinine glucose and levulose sugar tolerances and epinephrine response, van den Bergh, icteric index and Rosenthal tests and where possible, a bile drainage

These patients were then put on mono-iodo cinchophen two capsules three times daily. Five had the drug for seventeen weeks, four for nineteen weeks, six for twenty weeks, two for twenty-four weeks, and four for twenty-eight weeks. The average length of uninterrupted administration totaled twenty-

TABLE I—CONT'D

6	7	8	9	10	11
Arthritis deformans	Chronic hyper trophic	Arthritis deformans	Chronic hyper trophic	Chronic atrophic	Chronic atrophic
General	Knees, wrists spine	General	Hips, knees	Elbows, fingers	Knees
38 years	38 years	44 years	47 years	69 years	58 years
7 years	3 years	29 years	3 years	14 months	4 years
Tonsils, teeth	Teeth	Teeth, tonsils	Colon	Teeth, tonsils	Sinuses, mastoid
17 weeks	17 weeks	20 weeks	20 weeks	19 weeks	20 weeks
Neg	Neg	Neg	Neg	Neg	Neg
3950000	3970000	4000000	3700000	3780000	4000000
3930000	4020000	4090000	4110000	3760000	4390000
7100	5200	11500	8100	8000	7900
8600	7600	9100	8800	8100	7900
74	70	78	74	74	79
76	78	80	75	74	81
28.1	31	24.7	28	26.3	27
28.1	30.1	24.1	28.1	27	30
14	15.6	12.3	14.2	12.9	13.8
14.1	15	12	14.2	13.1	14.8
2.4	2.9	2.2	2.1	2.7	3.1
2.2	2.4	2.5	2.1	2.6	2.9
1.9	2	1.5	1.4	2.0	1.9
1.1	1.5	1.9	1.7	1.8	2.1
88.8	108.2	112.2	102.4	99.7	98.3
92.3	104.1	109.2	106.1	101.4	110.4
N	N	N	N	N	N
N	N	N	N	N	N
N	N	N	N	N	N
N	N	N	N	N	N
Neg	Neg	Neg	Neg	Neg	Neg
Neg	Neg	Neg	Neg	Neg	Neg
3.8	3.8	3.8	4.0	3.7	4.1
3.9	4.1	3.9	3.9	3.9	4.0
D- ID-	D- ID-	D- ID-	D- ID-	D- ID-	D- ID-
D- ID-	D- ID-	D- ID-	D- ID-	D- ID-	D- ID-
5% 15' 0 - 1 hr	3% 15' 0 - 1 hr	3% 15' 0 - 1 hr	4% 15' 0 - 1 hr	2% 15' 0 - 1 hr	4% 15' 0 - 1 hr
4% 15' 0 - 1 hr	4% 15' 0 - 1 hr	3% 15' 0 - 1 hr	3% 15' 0 - 1 hr	3% 15' 0 - 1 hr	5% 15' 0 - 1 hr
Not obtained	Not obtained	Neg	Neg	Neg	Neg
Not obtained	Not obtained	Neg	Neg	Neg	Neg
Neg	Neg	Neg	Neg	Neg	Neg
Neg	Neg	Neg	Neg	Neg	Neg
Alb neg crsts, pus cells	Neg	Alb crsts pus cells	Neg	Neg	Neg

one weeks. Some of these laboratory tests were carried out every week in order to catch any evidence of beginning trouble and all were repeated on the day on which the drug was discontinued and again one week and two weeks after the final withdrawal.

SUMMARY

Table I shows the results (1) of the examinations made before institution of treatment by mono-iodo cinchophen and (2) of the examinations made after withdrawal of the drug. At no time during the observation period did any of these patients show any variation from the normal.

TABLE I—CONT'D

CASES	12	13	14	15	16
Diagnosis	Semile atrophic	Arthritis deformans	Arthritis deformans	Arthritis deformans	Chronic hyper trophic
Involvement	Left hip	General	General	General	Sacro iliac, left ankle
Age	73 years	57 years	53 years	42 years	28 years
Duration	2 years	21 years	17 years	12 years	9 months
Foci	Colon	Teeth, tonsils	Tonsils, ethmoid	Teeth	Colon
Treatment period	17 weeks	24 weeks	28 weeks	28 weeks	24 weeks
W R	Neg	Neg	Neg	Neg	Neg
R B C *	3800000	4010000	4180000	3060000	4210000
	4680000	4350000	4270000	3350000	4480000
W B C	7800	6900	8100	7500	7600
	8400	8100	8600	8000	7800
Hb	75	78	78	60	74
	83	85	77	64	74
N P N	27.8	28.6	30.2	28.2	26.4
	26.8	28.8	28.2	29.7	30
Urea	13.7	14.4	14.4	13.4	13.2
	13.6	14.2	13.9	14.1	14.9
Uric Acid	2.1	2.4	2.3	2.7	2.8
	2.9	2.5	2.7	2.2	2.7
Creatinin	2.2	1.9	1.9	1.8	2
	1.9	2.1	1.8	1.9	1
Glucose	89.2	99.4	114.7	118.3	98.9
	92.4	107.2	110.1	100.6	107.5
Glucose tolerance	N	N	N	N	N
	N	N	N	N	N
Levulose tolerance	N	N	N	N	N
	N	N	N	N	N
Epinephrine response	Neg	Neg	Neg	Neg	Neg
	Neg	Neg	Neg	Neg	Neg
Icteric index	3.8	4.0	4.1	4.0	3.9
	4.0	4.2	4.5	4.4	4.0
Van den Bergh	D- ID-	D- ID-	D- ID-	D- ID-	D- ID-
	D- ID-	D- ID-	D- ID-	D- ID-	D- ID-
Rosenthal test	2% 15' 0 - 1 hr	4% 15' 0 - 1 hr	5% 15' 0 - 1 hr	2% 15' 0 - 1 hr	4% 15' 0 - 1 hr
	3% 15' 0 - 1 hr	4% 15' 0 - 1 hr	3% 15' 0 - 1 hr	2% 15' 0 - 1 hr	5% 15' 0 - 1 hr
Bile drainage	Neg	Neg	Neg	Neg	Neg
	Neg	Neg	Neg	Neg	Neg
Urobilogen	Neg	Neg	Neg	Neg	Neg
	Neg	Neg	Neg	Neg	Neg
Urine findings	Indican	Neg	Pus cells	Neg	Neg

All of the 21 patients recorded were advanced cases of arthritis with multiple involvements but were a group apparently without any lesions of the upper intestinal area. All took the drug without undue symptoms, some with improvement, some without, and this factor had apparently no influence on the findings. The drug was administered uninterruptedly in order that the results might be compared with cases of self-medication so common in the field of the arthritides.

Case 22 represents the original subject, self-treated for a period of considerably over six years. Two series of tests were made at an interval of six months.

While this group of cases is comparatively small, the length of time given over to the uninterrupted use of the drug is long, averaging five months, they are reported.

TABLE I—CONT'D

17	18	19	20	21	22
Arthritis deformans	Atrophic	Hypertrophic	Multiple infectious	Hypertrophic	Arthritis deformans
General	Hip	Shoulders, knees	Elbows, hands, knees, ankles	Lumbosacroiliac, elbows	General
67 years	58 years	42 years	23 years	39 years	52 years
28 years	8 years	3 years	9 months	4 years	17 years
Sinuses, ethmoid	Sinuses, ethmoid, sphenoid	Colon	Teeth, tonsils		
28 weeks	20 weeks	20 weeks	28 weeks	20 weeks	6 years, 9 mo (Cinchopen)
Neg	Neg	Neg	Neg	Neg	Neg
4050000	4210000	3710000	3650000	4130000	4010000
4110000	4180000	4220000	4260000	4180000	4090000
8200	8000	8000	6500	11200	7200
8100	8800	8900	8100	9100	7600
75	80	75	73	80	80
74	80	84	80	80	79
26 8	29 3	27 2	30	30	26 3
26 6	28 1	27 4	30 1	29 4	26 5
13 4	15	14 1	14 9	14 8	13 1
13 4	14 3	13 8	14 9	13 9	13 3
3 1	2 7	3 3	3 1	2 9	2 9
2 8	2 8	3 0	2 7	2 9	3 0
1 9	1 2	1 1	1 8	1 9	1 5
1 8	1 6	1 7	1 9	2 0	1 4
96 1	94 3	96 2	107 3	108 8	111 3
85 7	99 5	99 4	99 9	110 3	101 7
N	N	N	N	N	N
N	N	N	N	N	N
N	N	N	N	N	N
N	N	N	N	N	N
Neg	Neg	Neg	Neg	Neg	Neg
Neg	Neg	Neg	Neg	Neg	Neg
4 0	3 5	3 9	4	3 9	4 1
4 1	3 3	3 7	3 9	3 9	4 0
D- ID-	D- ID-	D- ID-	D- ID-	D- ID-	D- ID-
D- ID-	D- ID-	D- ID-	D- ID-	D- ID-	D- ID-
3% 15' 0 - 1 hr	4% 15' 0 - 1 hr	3% 15' 0 - 1 hr	4% 15' 0 - 1 hr	4% 15' 0 - 1 hr	4% 15' 0 - 1 hr
3% 15' 0 - 1 hr	3% 15' 0 - 1 hr	5% 15' 0 - 1 hr	4% 15' 0 - 1 hr	5% 15' 0 - 1 hr	4% 15' 0 - 1 hr
Neg	Neg	Neg	Neg	Neg	Neg
Neg	Neg	Neg	Neg	Neg	Neg
Neg	Neg	Neg	Neg	Neg	Neg
Alb, pus cells	Alb pus cells, casts	Indican	Neg	Neg	Alb, casts

1 As a method of possible safeguarding those persons using these drugs

2 As an effort to determine a reason for the apparent inconsistency in the appearance of toxic reactions from cinchopen and its congeners

In the one fatal case of cinchopen poisoning previously studied by the author there was a history of symptoms suggestive of liver and gall bladder disease antedating the taking of cinchopen by at least four years

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DIABETES MELLITUS AND THE GASTRIC SECRETION*†

BY MICHAEL G. WOHLE, M.D. PHILADELPHIA, PA

IN THE treatment of patients with diabetes mellitus there has been insufficient stress laid on the gastrointestinal tract. The high grade spastic obstipation is frequently commented upon, however, the not uncommon gastric complaints such as anorexia, feeling of distention, localizing discomfort after meals, and inability to consume the allotted amount of food have not attracted as much attention as they deserve.

Of 110 patients with diabetes mellitus, studied at the Mercy Hospital, Council Bluffs, Iowa, and at the Temple University Hospital, Philadelphia, 19 patients presented symptoms related to a disturbed gastrointestinal function.

The gastric contents were studied by the fractional method in 33 cases, 19 of whom manifested some gastrointestinal symptoms. Roentgen examinations were made in 12 patients and in 24 the stool was examined. In 10 the duodenal contents were obtained by means of the duodenal tube, and studied for the presence of pancreatic ferments.

At present I shall confine myself to the consideration of the gastric acidity. The group of patients comprised 21 females and 12 males.

The amount of sugar in the fasting blood varied from 160 mg per 100 c.c. of blood (the lowest) to 380 mg (the highest). Eighteen patients out of the 33 showed a hypoaclidity and achlorhydria. Eleven showed achlorhydria and seven hypoaclidity. Four patients had a hyperacidity. Eleven showed an acidity within the normal range. Two out of the hyperacidity group complained of a burning sensation after meals and of a marked constipation. Four out of the normal group had some gastrointestinal complaint. The patients were placed on proper diabetic diets, and insulin was administered when diet alone did not suffice to reduce the blood sugar to a level of 120 to 130 mg per 100 c.c. and when the urine still showed sugar. The patients with hypoaclidity and achlorhydria were given, in addition, dilute hydrochloric acid with each meal (5 c.c.). The gastric symptoms disappeared in 11 patients. Before the administration of dilute hydrochloric acid, they exhibited some gastric symptoms. Two had chronic cholecystitis, one of whom was operated upon and improved, one has not improved. (Table I includes a summary of these patients with achlorhydria and hypoaclidity.)

That the symptoms might be attributed to the achlorhydria can be deduced from the fact that no gastrointestinal pathology was demonstrated by the roentgen ray in 10 patients, then gastric complaints disappeared or dimin-

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†This study was commenced at the Mercy Hospital, Council Bluffs, Iowa, in 1925, since 1929 it has been continued at Temple University Hospital, Philadelphia, Pa.

ished after the administration of the dilute hydrochloric acid. I am aware of the fact, however, that achlorhydria and hypoacidity existed in five of the patients without any gastric symptoms.

TABLE I
CASES OF DIABETES SHOWING ACHLOPHYDRIA AND HYPOACIDITY

SEX	AGE	PROBABLE DURATION OF DIABETES	BLOOD SUGAR MG PER 100 C C	UPPING SUGAR PEP CENT	ACETONE AND DIACETIC	GASTRIC ANALYSIS
♀	50	6 yr	230	20	-	Free HCl ----- 0 Total acid max - 10
♂	40	3 yr	190	16	---	Free HCl ----- 0 Total acid max - 14
♀	45	4 yr	200	14	-	Free HCl ----- 0 Total acid max - 15
♀	48	6 yr	200	16	-	Free HCl ----- 0 Total acid max - 5
♀	50	8 yr	190	14	-	Free HCl ----- 0 Total acid max - 17
♀	55	8 yr	220	25	-	Free HCl ----- 0 Total acid max - 15
♀	48	12 yr	210	18	---	Free HCl ----- 0 Total acid max - 38
♀	46	7 yr	180	03	-	Free HCl ----- 0 Total acid max - 25
♂	65	15 yr	225	22	-	Free HCl ----- 0 Total acid max - 35
♀	54	12 yr	200	18	-	Free HCl ----- 0 Total acid max - 30
♂	50	11 yr	200	16	-	Free HCl ----- 0 Total acid max - 30
♂	50	8 yr	200	18	-	Free HCl ----- 5 Total acid max - 50
♂	46	3 yr	200	14	-	Free HCl max - 5 Total acid max - 30
♂	57	7 yr	280	24	-	Free HCl max - 18 Total acid max - 45
♀	35	2 yr	190	12	-	Free HCl max - 15 Total acid max - 38
♂	46	9 yr	210	23	-	Free HCl max - 12 Total acid max - 45
♀	58	11 yr	190	14	-	Free HCl max - 6 Total acid max - 20
♀	52	13 yr	185	12	-	Free HCl max - 12 Total acid max - 48

Knowledge of the gastric acidity in patients with diabetes mellitus may be of value in the interpretation of metabolism of carbohydrates. Glucose metabolism depends upon the activity of several factors. Among these, absorption (intestine), storage (liver) and utilization (tissues) (insulin is only one link in the chain) are of prime importance. Whether the rapid emptying of the stomach due to achlorhydria permits an unequal absorption from the intestine or some other obscure change incident to achlorhydria influences the fluctuation in blood and urine sugar is difficult to state at the present.

It is a clinical observation, however, that an individual who suffers from a functional hyperchlorhydria begins to show sugar in the urine once an achlorhydria develops. Explanations concerning the functional activity on the part of digestive organs and the blood sugar concentration are not lack-

ing We owe to Cammidge and Howard the credit for focusing our attention on the fact that the functional disturbances of gastric secretion play a part in carbohydrate metabolism

They are of the opinion that the alkaline secretion (pancreatic) below the pylorus which is initiated by the gastric hydrochloric acid causes a relative acidosis, they ascribe this to the abstraction of the bases from the blood for the formation of pancreatic secretion They have also shown that the entrance of alkaline bases into the circulation is accompanied by a lowering of the blood-sugar level, and vice versa an increase in the fixed acid of the blood causes a rise in the blood sugar The intimate relation between the acid base equilibrium and some phases of carbohydrate metabolism finds a measure of corroboration in the frequency with which hyperglycemia and glycosuria are worse in the early morning hours in some patients with diabetes Thus Leathes³ finds a high CO_2 of the alveolar air immediately upon waking, due to the accumulation of CO_2 in the blood during sleep This relative acidosis tends to disappear during the course of the morning, due to the reactivation of respiratory center with a fall of CO_2 in the blood Watson⁴ has suggested that the same process may be a factor in causing an increase in the blood and urine sugar during the early part of the day

Free hydrochloric acid is constantly absent in pernicious anemia The achlorhydria is generally regarded as a diagnostic criterion of pernicious anemia It is of interest in this connection to note that the fasting blood sugar in 16 patients with pernicious anemia was found by Johnson⁵ to be above normal Rennie⁶ performed glucose tolerance tests in 19 patients with pernicious anemia and found definitely abnormal tolerance curves in 58 per cent (11 cases), 8 of these having prolonged curves, 3 abnormally high The abnormality of the curves had no relation to the hemoglobin, red blood cell count age, weight, temperature, or pulse rate It would not be irrational to account for these curves by the abnormal physiologic activity of the digestive apparatus

Watson⁷ has obtained in 6 patients with achlorhydria sugar tolerance curves that speak for a disturbed carbohydrate metabolism In 3 of them an intermittent glucosuria was the only symptom suggestive of a defective carbohydrate tolerance

Dr D Meranze and I carried on some observations on nondiabetic hospital patients from Dr H B Shmookler's service of the Mount Sinai Hospital, that would tend to indicate the influence of the administration of hydrochloric acid on sugar tolerance curves Patients were selected who have shown an absence of free hydrochloric acid and who have shown no disturbance in carbohydrate metabolism One and seventy-five hundredths gm of glucose per each kilogram of body weight was given by mouth on empty stomach The glucose was dissolved in 400 cc of water and flavored with lemon juice Samples of blood for blood-sugar estimation were withdrawn in the fasting state and then every half hour for the first hour and two hours later Blood-sugar determinations were made by the Folin-Wu Micro blood-sugar method Two days later the same amount of glucose was given and in

addition the patient drank 5 cc of dilute hydrochloric acid with the glucose, and one hour later another dose of 5 cc of dilute hydrochloric acid was given. Table II illustrates the results of some of these experiments.

TABLE II
SUGAR TOLERANCE TEST

Fasting Blood	- - - - -	93	mg per 100 cc of blood
1 hr after ingestion of glucose	- - - - -	128	mg per 100 cc of blood
1 hr after ingestion of glucose	- - - - -	171.5	mg per 100 cc of blood
2 hr after ingestion of glucose	- - - - -	99	mg per 100 cc of blood

CASE 4—Mr M T. Free HCl 0, total acidity max 15
Glucose plus Hydrochloric Acid (5 cc)
Another 5 cc was given at end of first hour

Fasting Blood	- - - - -	120	mg per 100 cc of blood
1 hr	- - - - -	248	mg per 100 cc of blood
1 hr	- - - - -	278	mg per 100 cc of blood
2 hr	- - - - -	171	mg per 100 cc of blood

SUGAR TOLERANCE TEST

Fasting Blood	- - - - -	80	mg per 100 cc of blood
1 hr after ingestion of glucose	- - - - -	111	mg per 100 cc of blood
1 hr after ingestion of glucose	- - - - -	129	mg per 100 cc of blood
2 hr after ingestion of glucose	- - - - -	120	mg per 100 cc of blood

CASE 7—Mr J W. Free HCl 0, total acidity max 20
Glucose plus Hydrochloric Acid (5 cc)
Another 5 cc was given at end of first hour

Fasting Blood	- - - - -	110	mg per 100 cc of blood
1 hr	- - - - -	218	mg per 100 cc of blood
1 hr	- - - - -	206	mg per 100 cc of blood
2 hr	- - - - -	185	mg per 100 cc of blood

Comment Achlorhydria has been found in from 4 to 6 per cent of apparently normal persons.⁵ This agrees with the work of Bennett and Ryle,⁶ who in 100 medical students of an average age twenty years, demonstrated a complete absence of free hydrochloric acid in four persons. Other investigators Lockwood (quoted by Alvarez⁵) Eggleston¹⁰ encountered achlorhydria in from 6 to 10 per cent of normal cases.

The occurrence of achlorhydria and hypoaclidity in diabetes mellitus is of greater frequency than normally found. In our study, achlorhydria occurred in about 33.3 per cent of the cases and hypoaclidity in 21.2 per cent. That occurrence of achlorhydria in diabetes mellitus has not been given its proper valuation may be gleaned from Faber's¹¹ work. He states that of all achlorhydria individuals 10 per cent develop pernicious anemia and 90 per cent may suffer from exophthalmic goiter, arthritis deformans, cholecystitis. He fails to mention diabetes mellitus.

The relative frequency of achlorhydria and hypoaclidity is indicated in Table III.

Bowen and Aaron,¹² and Joslin¹ found achlorhydria in 27.3 per cent of cases. The incidence of achlorhydria in diabetes including this group is 28.7 per cent. A prominent symptom in Bowen and Aaron's patients was the diarrhea. According to them diarrhea was not noted unless achlorhydria

TABLE III

GASTRIC ACIDITY IN DIABETES ACCORDING TO THE AGE OF PATIENT AND DURATION OF DISEASE

NUMBER OF PATIENTS	DURATION OF DISEASE	AVERAGE AGE	SEX		FREE HYDROCHLORIC ACID		
			MALE	FEMALE	NORMAL OR ABOVE	DIMIN- ISHED	ABSENT
Bowen and Aaron							
46	Under 5 years	49	16	30	29	8	9
16	From 5 to 10 yr	53	3	13	4	6	6
4	From 10 to 15 yr	50	2	2	1	0	3
2	From 15 to 20 yr	66	1	1	1	0	1
1	From 20 to 25 yr	64	0	1	0	0	1
Joslin							
10	Under 5 years	46	4	6	8	0	2
10	From 5 to 10 yr	53	3	7	6	1	3
7	From 10 to 15 yr	55	1	6	5	0	2
5	From 15 to 20 yr	58	1	4	4	0	1
5	From 20 to 25 yr	55	3	2	4	0	1
Wohl							
11	Under 5 years	45	4	7	7	2	2
14	From 5 to 10 yr	51	5	9	6	3	7
8	From 10 to 15 yr	54	3	5	2	2	4
139			46	93	77, or 55.4%	22, or 16%	40, or 28.7%

existed. According to our study diarrhea was not a frequent symptom. Of the 11 patients with achlorhydria, one patient complained of diarrhea and in another was an alternating diarrhea and constipation. In the patient with diarrhea this was checked by the administration of dilute hydrochloric acid. In the second a colitis was demonstrated by sigmoidoscopic examination. It is interesting to note that the patients showing achlorhydria had diabetes of a long duration. The known causes for achlorhydria and hypoacidity such as cholecystitis, tuberculosis, carcinoma of the stomach and pernicious anemia have not played a rôle in our patients, as in only two patients with achlorhydria there was demonstrated a chronic cholecystitis.

The relationship between the chloride metabolism and the secretion of free hydrochloric acid in the stomach is well established. It is significant that in diabetes one frequently finds a diminution of chlorides in the pancreatic secretions. Meyer-Bish¹⁴ has noted in diabetes a close parallelism between the reduction of pancreatic ferments and the chlorides in the pancreatic juice.

Lee Foshay¹⁵ has also observed a reduction of the serum chloride concentration with a corresponding increase of the chloride in the blood corpuscles when the blood sugar was increased, in other words in diabetes the chloride is in a less available form for formation of hydrochloric acid by gastric glands than in health.

CONCLUSIONS

In the treatment of patients with diabetes mellitus attention should be given to the gastrointestinal tract. In a respectable number of patients an achlorhydria and hypoacidity may be demonstrated.

The administration of dilute hydrochloric acid in addition to the diabetic regimen may lessen the gastric complaints in such persons.

There is some clinical and laboratory evidence of the relationship of a disturbed gastric function to disturbed carbohydrate metabolism. It suggests itself that in the interpretation of abnormal sugar tolerance curves one should also consider the physiologic function of the digestive tract.

I wish to express my appreciation to Dr Wm Egbert Robertson for his many valuable suggestions in the preparation of the work and to Dr Herman Jahr of Omaha, Nebraska and Dr D Meranze for technical assistance.

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- NOTE Since the preparation of the manuscript an article appeared by Howard F Root Diabetes and Pernicious Anemia *J A M A* 96 March 21, 1931, dealing with the incidence of Achlorhydria in Diabetes Mellitus

SEPTIC CAVERNOUS SINUS THROMBOSIS*

REPORT OF TWO CASES WITH RECOVERY OF ONE FOLLOWING BACTERIOPHAGE THERAPY

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ACUTE infectious cavernous sinus thrombosis is a relatively rare disease if one considers the number of cases reported in the literature. However, it is possibly more common than these reports show cases occurring which are either not reported or not recognized as such.

The most extensive treatise on this subject is the book by Eagleton¹ who in 1926 collected the reports up to that time and added in detail 25 cases of his own. Smith,² in 1918, reviewed the literature and found less than 300 cases reported up to that time. Dixon,³ in 1926 reported 10 cases one with recovery, but which he regarded as an error in diagnosis.

Regarding the prognosis Dwight and Germann,⁴ in 1902, collected 182 cases from the literature and found that 7 per cent of these patients recovered spontaneously. Babbit,⁵ commenting on this, stated "that 7 per cent recoveries must raise the question of diagnosis without autopsy." Smith² expressed the opinion that "on the assumption that the clot in the sinus was not infected and considering the great number of observers, the fragmentary character of many of the reports, and the possibilities of maceracies and of errors in diagnosis, we are forced to the conclusion that thrombosis of the cavernous sinus is practically always fatal if the thrombus is infected and not drained." Dixon concluded "that meningitis follows so closely after the eye symptoms develop that septic thrombosis of the cavernous sinus is a non surgical complication and that reported cases of recovery were probably errors in diagnosis."

This paper has to do with the report of two cases, the first with classical symptoms, which recovered completely without sequelae and in which radical surgery was not employed, but dependence placed on the use of antistaphylococcus bacteriophage filtrates and blood transfusions. A second case, also typical, but which came under observation too late for any hope of recovery.

CASE 1—Patient, L. D., female, aged sixteen. Family history negative. Past history. Perfect health up to the time of the present illness. On July 19, 1930, a small furuncle appeared on the center of the chin just below the border of the lip and was treated by local application of hot fomentations. On July 23 it was lanced, little pus being found. The lip began rapidly swelling and she entered the Medical Arts Hospital on the next morning with a temperature of 101.2° which rose to 103.2° the same day. One cc. of bacteriophage was injected under the skin and a wet dressing of it used locally. On the following day the lesion was cauterized deeply with the thermocautery and the bacteriophage

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treatment reported. On the following day, July 26 (the seventh day), the swelling had rapidly spread up the right side of the face, with continued high temperature to 103.6° . Because of the cellulitis with no pus forming in the lesion, 50 c.c. of antistreptococcus serum was given in the belief that it was a streptococcus infection.

On July 28 (the ninth day) the first blood count was made showing a low total leucocyte count of 7500 but with 96 per cent neutrophiles, 57 per cent of which were immature forms constituting a dangerous left nuclear shift. A blood culture and cultures from the lesions were made. All showed *Staphylococcus aureus*, the blood in one c.c. amounts in 30 c.c. tubes of broth yielding a heavy growth in ten hours. By the next morning (the tenth day) swelling was intense over the right side of the face, involving the right eye which showed exophthalmos and chemosis. The right side of the neck was also brawny and hard. The temperature rose to 103.4° and the blood continued to show a most dangerous picture.

By July 31 (the thirteenth day) the left eye was involved but to a less extent than the right, which showed now extreme exophthalmos and chemosis. The thrombus had invaded the left side of the sinus. The lids were swollen shut, were a bluish purple, pitted on pressure and could not be opened enough to permit an examination of the eyegrounds. The tension was so great that pressure necrosis was feared, and it was decided to make superficial linear incisions in the lids and conjunctiva to relieve the tension. This was done together with a small incision in the swollen cheek. No pus was found. Reference to Table I will show the extreme gravity of the blood picture from day to day, the temperature range, and the therapy used.

Bacteriophage was at first used subcutaneously and locally, but in view of the heavy blood stream infection, in addition to the rapid increase in symptoms, it was decided to use it intravenously. This was done daily with doses morning and afternoon on three of the days. July 29 (the tenth day) 4 c.c. were given subcutaneously and on the thirtieth, 9 c.c. in divided doses were given under the skin. The intravenous treatment was well borne, being at first followed by short but definite chills, those later becoming mild. On August 3 (the sixteenth day) urticaria with painful joints developed and because the intravenous injections of bacteriophage greatly aggravated these symptoms, it was abandoned. The urticaria was undoubtedly caused by the antistreptococcus serum which was given on July 26. A blood culture taken at this time and cultured both in broth and on agar plates remained sterile after seven days incubation.

Reference to Table I will show the number of blood transfusions and the use of parathormone and calcium given for the control of the urticaria and other therapy. The total leucocyte count began to rise though the neutrophiles and nuclear shift did not show much improvement. An occasional eosinophile appeared which may have been due to urticaria.

By August 6 (the nineteenth day) the swelling in the neck and face had begun to improve and the wound in the lower lid showed signs of healing as did the superficial incisions in the eyelids. The exophthalmos and chemosis in both eyes was still marked but the lids could now be separated for examination of the eyegrounds. The pupil of the right eye was somewhat dilated but the ophthalmologic examination showed nothing but congestion of the retinal veins while the nerve and macula appeared normal in both eyes. The patient was still unable to voluntarily raise the lids of either eye. From this time until August 22 the swelling except for bulging of the eyes had subsided completely, with healing of the incisions. The patient's mind was clear and her appetite good. However, the blood picture showed higher total counts of the leucocytes, a marked left nuclear shift and she had occasional delirium when asleep.

The temperature was somewhat lower in range but headache with pain in the back of the head grew increasingly severe until on August 23 (the thirty second day) she became very ill with vomiting and severe pain in the back of the head and neck. The leucocytes rose to 20,000 and the temperature to 103.2° . At 4:00 P.M. a lumbar puncture was made yielding 15 c.c. of a turbid fluid under high pressure. The cell count showed 1100 per c.mm., while direct smears and cultures showed numerous staphylococci. One c.c. of bacteriophage was introduced into the spinal canal. Since I had no precedent for the intraspinal use of bacteriophage I decided on 1 c.c. as a trial dose. A blood culture taken at this time

TABLE I
Case No 1

DATE	HEMOGRAM										TEMPERATURE RANGE 24 HR	BACTERIOLOGICAL TITRATION	OTHER MEDICATION	REMARKS
	HEMOGLOBIN PER CENT	RED CELLS IN MILLION	WHITE CELLS	% = 0 MYE	% = 0 LYM	% = 2.5 NEUT	% = 2.5 EOS	% = 1 BAS	% = 20.30 LYM	% = 6.9 MON				
7/24/30											101.8 to 104.2	1 cc subcutaneous local application	100 cc intravenous streptococcus serum	Entered hospital by much swollen
7/25/30											100 to 104.6	1 cc hypo	Thrombin	Increasing severity of swelling
7/26/30											101.2 to 105.6	50 cc intravenous	100 cc intravenous	Swelling spreading up side of face
7/27/30											102 to 104.1	1 cc hypo		Right eye beginning to be involved
7/28/30		7,800		5	12	39	0	0	3	1	101.2 to 105	1 cc hypo	300 cc glucose (25 per cent) intravenously	Culture made from blood and lesions. Increasing swelling of right eye
7/29/30	80	8,000		3	13	38	0	1	6	3	99 to 103.4	15 cc intravenous hypo	Blood transfusion 250 cc	Blood cultures show growth in 10 hr. Pseudomonas chemosis ophthalmos. chemosis swelling of face and neck. Chill followed phage injection
7/30/30		6,700		15	17	20	0	0	1	4	100 to 103.4	15 cc intravenous phage	Blood transfusion 250 cc	Light chill followed intravenous phage
7/31/30		9,700		10	15	33	0	0	5	1	101.8 to 104.2	15 cc intravenous divided doses and 1 cc hypo		Left eye involved, in crusts made in lids and cheek

TABLE I—Cont'd

DATE	HEMOGLOBIN PER CENT	RED CELLS IN MILLIONS	TOTAL WHITE CELLS	HEMOGRAM NORMALS								TEMPERATURE RANGE 24 HR.	BACTERIOLOGIC THERAPY	OTHER MEDICATION	REMARKS
				% = 0	% = 0	% = 0	% = 0	% = 0	% = 0	% = 0	% = 0				
				MYL	JUV	% = 0	% = 0	STABS	% = 60-70	TOTAL LEUCOPHILES	% = 3-5	% = 1	% = 20-30	LYMPH	% = 6-8
8/15/30	80	4.14	13,600	0.5	2.5	28	52.5	81.5	5	0	11.5	99	to 103.8		Pain in back of neck and right ear
8/16/30	70	4.30	13,750	0.5	2.5	29.5	49	81.5	1	0	10.5	99	to 101		Pain in back of head and neck. General body soreness.
8/18/30	75	4.55	13,500	0.5	0	20	58	78.5	0	0	11.5	99	to 100		Swelling in face almost gone, lip normal and eye incisions healed.
8/22/30	72	3.75	15,500	0	1	17	65.5	83.5	0.5	1	13	101	to 102.4	Codene and aspirin	Pain in head and neck. General body soreness.
8/23/30	73	3.74	20,000	1	3	27	53	78	0	0	11	101.2	to 103.2	Codene and aspirin	Severe pain in back of head and neck, nausea and vomiting. Lumbar puncture. Turbid fluid. Cell count 1100. Staph found smears and cultures.
8/24/30	77	4.30	13,400	0	0	9	76	85	0	0	6	99.8	to 101.2	Codene and aspirin	Pain much less. Nausea less. Spinal fluid shows 170 cells. Blood culture sterile.
8/25/30	76	4.36	11,400	1	1	20	66	88	2	0	9	98	to 99.6	Codene	Cell count of fluid was 320, culture showed one colony on plate. Less headache, sleeping much.
8/26/30	70	3.76	12,000	1	1	12	72	86	1	0	7	97.4	to 99	Codene	Cell count of fluid was 276. Mind clear, sleeps but still pain in head.

gave no subsequent growth. The next morning at 9:00 A.M. the blood count showed 13,400 leucocytes. The shift was more to the right and the patient felt considerably better. At 11:00 A.M. a lumbar puncture was made, the fluid being less under pressure while the cell count showed now 370 cells. Since no reaction followed the 1 cc dose, 4 cc of bacteriophage were injected into the spinal canal. The temperature rose only to 101.4° following this. Lumbar punctures were made daily for the following four days, each time 7 cc of bacteriophage being introduced after the fluid was withdrawn. Only 10 cc of fluid was withdrawn because of the fear of disturbing the lesions above.

The patient improved rapidly, sleeping much of the time, the appetite returned, but she complained continually of headache, which was controlled by codein. The temperature dropped close to normal after the second intraspinal injection of bacteriophage and the blood picture improved. On September 3 vomiting and pain in the head and right eye returned, however, the temperature range was not high, and the blood picture was nearly normal. Another lumbar puncture was made, showing a clear fluid under normal pressure and with a cell count of 16. Cultures from the fluid yielded no growth after seven days' incubation. Again 7 cc of bacteriophage were given intraspinaly. The temperature rose to 101.4°. The urine, which had been examined daily since the patient's entrance to the hospital, at this time showed a large amount of pus with staphylococci and B. coli. On September 4 a cystoscopy and ureteral catheterization was done, the urine from each kidney showing pus with staphylococci and B. coli. Mixed staphylococcus and coli bacteriophage was introduced into the kidney pelvis. Following this the urine showed variable amounts of pus from 2 to 4 plus. Local bladder irrigations and instillations were made daily.

Bacteriophage treatment by subcutaneous injection was resumed on September 13 (the fifty-third day) and was followed by no reaction of any kind. The blood picture from day to day showed an increasing leucocytosis, a variable neutrophilia and a marked left nuclear shift. The patient hid, of course, rapidly lost weight from the first and by now was emaciated and extremely weak. The eyes, following the intraspinal injections of bacteriophage had rapidly improved with ability to voluntarily raise the lids, the exophthalmos subsided, vision was good with the exception of diplopia, and the patient was in good spirits. The temperature reached nearly normal in the mornings but rose each afternoon. On September 15 (the fifty-fifth day) the temperature rose to 103.8°, the leucocytes to 17,350 and nausea and vomiting with headache returned. A lumbar puncture yielded a clear fluid under normal pressure with a count of 21 cells. Cultures of the fluid were again sterile. Another blood culture was made which gave no growth. Two cc of bacteriophage were injected into the spinal canal.

The urine at this time showed a 4 plus pus with B. coli but the fear that the infected sinus was still causing the symptoms, in spite of negative spinal fluid findings, was so strong that treatment of the kidney pelvis was not done until October 1 (the seventy-first day). On this day nausea reappeared with chills sensations, the total leucocytes rose to 22,800 though the neutrophiles were only 73 per cent and the nuclear shift not marked.

The ureters were catheterized, pus being found from the right ureter, and argyrol was injected into the kidney pelvis. Following this the temperature dropped to normal and remained so until the patient was discharged from the hospital on October 8 (the seventy-eighth day). No further blood counts were made, the eyes were nearly normal and the pus content of the urine dropped to a few cells. After the patient returned home she had a few slight rises in temperature with some congestion about the eyes, these attacks being coincident with her menstrual periods, and by the first of December she weighed ten pounds more than before her illness and had normal vision and no remaining effects of the infection.

CASE 2—Patient, Mrs. K. C., aged thirty-four, entered the Robert B. Green Memorial Hospital September 18, 1930, at 2:00 P.M. Service of Dr. Dudley Jackson.

Past History. During past three months she had recurring attacks of furuncles about the face. September 11 she had a small one on the left lower lip. On the fourteenth (third day) she picked it, and the next day the face and lip began swelling, increasing in severity until she entered the hospital where examination showed the whole left side of the face to be swollen, including the left eye which she was unable to open. The lids were

edematous and bluish red in color. There was chemosis and a small amount of exophthalmos present. She complained of severe pain in the head and showed much mental confusion. The temperature rose from 100.8°, on entrance, to 104.4° in the night. On the following day (the fourth day) the patient became delirious with increase in the swelling of the face and with chemosis present in both eyes.

A lumbar puncture showed a slightly turbid fluid with 72 cells and a few staphylococci on direct smear. The leucocytes were 16,400 with 77 per cent of neutrophils (the Schilling count was not made). At 2:00 P.M. 2 c.c. of antistaphylococcus bacteriophage were given subcutaneously. The patient continued to grow rapidly worse during the night, and the following morning, September 20 (the ninth day) 2 c.c. of bacteriophage were given subcutaneously. Following a lumbar puncture at 2:30 P.M. which showed a purulent fluid, 1 c.c. of phage was given intraspinally. The temperature rose to 104°, the pulse to 140 and at 4:00 P.M. she died, having been in the hospital about forty-eight hours.

At autopsy (Dr. Dudley Jackson), there was found on opening the skull, a general meningeal exudate with a collection of purulent material in the region of the left frontal lobe of the brain. Both orbital cavities were filled with an infected edematous material with thrombosis of the orbital veins. The longitudinal sinus contained an infected thrombus and on opening the cavernous sinuses both were found filled with a purulent soft clot. The pleural cavities on both sides contained a seropurulent exudate, while the lungs were filled with infected emboli of various sizes from small areas to larger consolidations. The pus from the cavernous sinuses and other lesions yielded pure cultures of *Staphylococcus aureus*. The other organs of the body showed nothing of importance.

DISCUSSION OF CASE I

This case report presents several outstanding points of interest and importance.

First that a patient with a classical picture of infectious cavernous sinus thrombosis with septicemia and meningitis should survive at all.

Second the sterilization of a heavy blood stream infection by intravenous bacteriophage therapy.

Third the control of a *Staphylococcus meningitis* by intraspinal use of bacteriophage.

Fourth the faithfulness with which the Schilling index of the leucocytes mirrored the clinical facts in the case.

The chief disease simulating cavernous thrombosis is orbital cellulitis. Eagleton¹ and Babbitt have discussed this question and pointed out the differential points in diagnosis. In orbital cellulitis the disease nearly always starts in the nasal sinuses or from direct trauma to the eye. The serious blood picture in this case persisting in spite of the subsidence of external signs of infection with the exception of the eyes indicated with certainty the presence of a focus somewhere in the brain or venous sinuses. The appearance of delirium during the days preceding the development of meningitis as shown by the spinal fluid and the severe headache according to Eagleton¹ do not appear when the infection is confined to the sinus. He states that "the appearance of delirium is of the gravest significance." The lungs and endocardium at no time showed any involvement and no other metastatic foci developed during the illness.

Concerning the question of spontaneous recovery reference to what has been said about prognosis in this paper together with the history of an infected sinus thrombosis, septicemia and meningitis casts grave doubt as to

its possibility in this case. In the treatment of this patient, aside from six blood transfusions and other supportive measures, the one hope was centered on the specific treatment by antistaphylococcus bacteriophage. Faith that this might be of aid in spite of the desperate nature of the case was inspired by previous experience with less alarming yet severe staphylococcus infections.⁶

In this case the infection progressed so rapidly and with such alarming symptoms, including a blood picture that showed an overwhelming toxic effect on the bone marrow that the first attempts to cope with the infection by subcutaneous and local use were soon abandoned for intravenous injections. These were at first followed by definite chills but later injections were well borne until the severe urticaria and joint pains forced the suspension of bacteriophage therapy for the time. The urticaria was undoubtedly due to the previous use of the antistreptococcus serum and was aggravated by the bacteriophage injections. However, following six days of intravenous bacteriophage therapy a blood culture showed the blood to be sterile.

Lowenstein⁷ has quoted Stetson as saying that the staphylococcus is the deadliest organism encountered in general sepsis, also that Peet Reed and Stiles and others have stressed the almost invariably fatal outcome of Staphylococcus septicemia when secondary to furuncles. He reported one case of encephalitis in which Staphylococcus albus was isolated from both the blood and spinal fluid. The patient recovered following intramuscular, intravenous and intraspinal injections of staphylococcus antitoxin. However, in two other patients treated by this method, the first died on the day after entering the hospital, while in the other, 7 intravenous injections failed to have the slightest effect in preventing the fatal termination. He called attention to the fact that the staphylococcus is very irregular in the production of soluble toxin upon which dependence must be placed for the production of antitoxin. In two of his cases he used nonspecific protein without success and commented on the little encouragement given in the literature to this form of therapy.

Rice,⁸ in reporting two cases of Staphylococcus septicemia, stated that both died, though one lived weeks longer than was expected. Local and subcutaneous treatment by bacteriophage was employed, fear of the peptone in the filtrate preventing the intravenous use of it.

The early sterilization of the blood stream in this case, I believe, prevented the development of metastatic foci in the lungs which are so frequently found, as well as elsewhere. It also localized the infection and confined it to the original sites. This action of bacteriophage in less serious infections has been noted by numerous observers as a very common and characteristic result. I have seen numerous cases of severe cellulitis of the face arising from foci about the mouth or nose in which local and subcutaneous use of the bacteriophage caused rapid subsidence of the cellulitis with localization, relief of pain, and prompt termination of the infection. The infection in this case was obviously confined to the sinuses, with a local meningitis, until later when it became generalized. The most spectacular evidence of the rôle of bacteriophage in the recovery of this patient was shown by the rapid and complete control of the meningitis by the intraspinal method of administration.

In both the intravenous and intraspinal methods direct contact was assured with the organisms causing the infection constituting the ideal method of approach. While the peptone in the filtrate may be an obstacle to the use of large amounts intravenously, the harmless nature of this agent used locally is such that the entire meningeal cavity could probably be filled with it, replacing the spinal fluid.

The therapeutic value of antistaphylococcus bacteriophage filtrates has been much in dispute. However such favorable reports have been published by d'Herelle,⁹ Bruvnotte and Maisson,¹⁰ Gratia,¹¹ Gougerat and Payre,¹² Haudumov,¹³ Grenet and Isaac-Geroges,¹⁴ Bazv,¹⁵ Lingeman,¹⁶ Raiga,¹⁷ Rice,¹⁸ Larkum,¹⁹ Alderson,¹⁹ Clutchfield and Stout⁶ and others, that the mass of evidence supporting the therapeutic value of this agent cannot be ignored.

D'Herelle⁹ states that "too much emphasis cannot be placed on the fact that bacteriophage acts effectively only when very virulent races are used." Nelson²⁰ has studied the effect on phagocytosis by injecting rabbits intravenously with 5 c.c. of bacteriophage together with a strain of staphylococcus susceptible to lysis and found an immediate and marked increase in the phagocytic index. With a strain of staphylococcus not lysable the phagocytic index was not altered. Smith²¹ has shown in a similar study that the degree of phagocytosis is determined not only by the period of contact with bacteriophage but also by the virulence of the lytic principle employed and that the bacteria become more susceptible to phagocytosis after contact has been interrupted. D'Herelle,⁹ Gohs and Jacobsohn²² and others, have also demonstrated the marked effect on phagocytosis. Arnold and Weiss²³ found that a single dose of bacteriophage lysed bacterial filtrate developed a rapid increase in the antibody titer of the rabbit and was able to protect the animal from a lethal dose of the homologous organism. The bacteriophage lysed organisms being split products were thus shown to have a more rapid and active antibody forming power than vaccines or autolysates. Vaccines have been regarded as helpful but not curative in *Staphylococcus* septicemia and then only in chronic cases.

The race of bacteriophage used in this case was one of high virulence having a titer of 10^{-5} and has been capable of lysing more than 90 per cent of the strains tested. The strain recovered from the lesions of the patient was readily susceptible to its action. It is my belief that this patient survived only because of early and vigorous treatment, particularly by the intravenous method. In giving this patient only 1 c.c. doses subcutaneously during the first five days much time was lost. Much larger amounts should have been used, given twice or three times daily and earlier intravenous treatment instituted.

If confronted with a case such as Case 2 in which meningitis has become generalized and septic emboli have already invaded the lungs no hope can be entertained of recovery by any form of treatment. This patient had been subject to staphylococcus infection which lowered her resistance and rendered her more vulnerable to the final invasion of vital structures. Had she been fortunate enough to have been seen early and vigorous and fearless bacteriophage treatment been instituted she probably would have survived.

Reference to the history of Case 1 will show how narrow a margin of chance the patient had during the first days of the infection. The repeated injections subcutaneously of large doses together with the intravenous dosage produced such a resistance that the breaking through of the infection into the meninges did not take place until more than three weeks after the bacteriophage therapy began. It would seem clear that the study of the behavior of this patient under bacteriophage treatment should teach more than that one case of its type has recovered following its use. These cases are uncommon, but it is well known that staphylococcus infections about the face, lips or nose are common, and all potentially dangerous. These can be promptly and certainly relieved by early and adequate bacteriophage treatment.

The success in sterilizing the blood stream in this case should open a field of usefulness in *Staphylococcus septicemia* from other foci. It should of course be combined with such surgery as seems indicated in each case and should be used locally subcutaneously and intravenously. Doses up to 5 c.c. at a single injection can be used subcutaneously, while from 1 to 2 c.c. intravenously were safely employed in this case. Nelson²⁰ used daily intravenous doses in rabbits of 5 c.c. without apparent harm.

It is hoped that the success attending the use of bacteriophage in Case 1 will stimulate further study and experiment in this class of infections and lead to the saving of some at least of these otherwise hopeless cases.

I am indebted to the following physicians for permission to report Case 1. This case was under the direct charge of Drs. O. H. Timmins and John B. Herff, consultants, but in daily attendance, were Dr. Herbert Hill, Internist, Dr. C. F. Lehmann, Dermatologist, Drs. Charles Boelis and W. D. Hicks, Ophthalmologists, Drs. Max Johnson, C. E. Scull, Dudley Jackson, general surgeons and Drs. R. R. Ross and W. H. Heck, Urologists. I was intrusted with the laboratory studies, the preparation of the bacteriophage, and the direction of its use. Case 2 was under the care of Dr. Dudley Jackson who gave permission to include it in this paper.

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THE GONOCOCCUS COMPLEMENT-FIXATION TEST IN SYNOVIAL FLUID

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THE value of the complement-fixation test in the blood serum for diagnosis of gonorrheal arthritis is recognized by all investigators. Our study presents this reaction in the synovial fluids of arthritis of different etiology and stage.

TECHNIC

We have used the polyvalent antigen manufactured by the Lederle Laboratories according to the principles of Teague and Torrey. Experiments with various amounts demonstrated that 0.1 c.c. of synovial fluid was the optimum to secure specific reactions. Inactivation of the synovial fluid was found to be necessary to prevent anticomplementary reaction. The fluids must be perfectly clear; it has been found that even slight turbidity or hemolysis have interfered with the results. The following routine was used.

The synovial effusion was centrifuged at high speed until the clear fluid is separated from the sediment. Coagula were detached from the walls of the test tubes by means of a glass rod. The clear fluid is inactivated from fifteen to thirty minutes in a water bath at 56° C. To 0.1 c.c. of synovial fluid in a small test tube there is added 0.1 c.c. of 1:10 dilution of gonococcus antigen and the amount of complement determined by titration together with 0.5 c.c. of normal saline. The tubes were then shaken and incubated in the water-

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bath at 37° C for one hour, after which they were placed in the ice box for three hours. Five-tenths c.c. of 5 per cent suspension of sheep cells and 1 unit of amboceptor were added. The whole was incubated for one hour and readings were made. Controls, positive negative and hemolytic, as well as anticomplementary, were run with each test. Complete fixation is marked four-plus, three-, two- and one-plus signify degrees of partial fixation, complete hemolysis is marked negative. Fluids should be preserved in the ice box and tests carried out as soon as possible.

ANALYSIS OF THE MATERIAL

The reaction was carried out on 121 cases. Fifteen fluids were aspirated from traumatic and 106 (87.6 per cent) from inflammatory effusions. Of the latter 36 (33.9 per cent) were from acute cases and 70 (66.1 per cent) were from chronic conditions (duration over six months). Of the 106 inflammatory fluids, 95 (89.6 per cent) gave a negative reaction and 11 (10.4 per cent) a positive reaction. Of these 11 positives, 7 were aspirated from knee joints, 2 from elbows, 1 from the shoulder and 1 from the ankle. The reaction was four-plus in 6 fluids, three-plus in 1 fluid, two-plus in 3, and one-plus in 1 fluid. Of the 36 acute effusions, 8 (22.2 per cent) gave positive results. Of the 70 chronic cases, 3 (4.3 per cent) gave positive reactions.

SPECIFICITY OF REACTION

a The Wassermann reaction was carried out simultaneously in all, and was positive in 16 fluids. Of these 13 cases had a negative gonococcus complement-fixation test. In 3 fluids both reactions were strongly positive. The history in 2 cases showed an old syphilitic and a recent gonorrheal infection.

CASE 1—(No. 4 on Table I) J. McD., twenty three years old. Contracted gonorrheal urethritis three months previously, followed by epididymitis. Two months ago pain in left wrist and toes. At the time of his admission to the clinic he had a swelling of the left wrist and right knee joints. Sixty c.c. of turbid fluid containing 26,100 white cells per c.mm. with 86 per cent polymorphonuclear leucocytes were aspirated. The Wassermann and gonorrheal fixation tests were four plus in both blood and fluid. Culture of the synovial fluid gave no growth. However, gonococci were found in a smear of the urethral discharge.

CASE 2—(No. 1 on Table I) C. B., twenty nine years old. Contracted gonorrhea four weeks previously and for two weeks the right ankle had been swollen and tender. Three c.c. of a turbid fluid containing numerous polymorphonuclear leucocytes were aspirated. The Wassermann and gonococcus complement fixation tests gave a four plus in the fluid and blood serum. Culture of the synovial fluid was negative but gonococci were found in direct smear from the synovial fluid.

CASE 3—(No. 6 on Table I) L. W., colored female, thirty one years old. Pain and swelling of wrists and sterno clavicular joints and effusion of both knees for about a year. Ptosis of the right eyelid. The aspirated fluid was turbid with a white count of 1,800 per c.mm. and 46 per cent polymorphonuclear leucocytes. Wassermann and gonococcus fixation tests in the fluid were four plus, Wassermann in the blood was four plus, gonococcus in the blood was two plus.

b Thirteen fluids were inoculated into guinea pigs, of these 4 developed tuberculosis. None of the fluids gave a positive gonococcus complement-fixation test. However, one fluid obtained at operation showed a two-plus

gonococcus reaction while the histologic examination of the synovial membrane revealed tubercles

TABLE I*

NO	NAME	DURATION GONOP PHEA	DURATION ARTHRITIS	UPOGENITAL GONORRHEA	GNOCOCCUS COM- PLEMENT FIXATION		REMARKS
					SYNOVIAL FLUID	BLOOD	
1	CB	4 wk	2 wk	Urethral epididymitis	++++	++++	Wassermann +---, gonorrhea positive in sediment of synovial fluid and discharge of urethra
2	OK	8 wk	5 wk	Urethral and prostatitis	----	Not done	
3	DK	11 wk	1 wk	Prostatitis	+++	+++	
4	J McD	15 wk	8 wk	Epididymitis	++++	++++	Wassermann +---, gonorrhea positive in discharge
5	ER	2 yr	6 wk	Prostatitis	+++	----	
6	LW	"	1 yr	"	+++	++	Wassermann +---
7	MA	2 1/2 yr	3 wk	"	+++	—	First attack 2 years ago
8	T	"	2 wk	"	++	Not done	
9	SW	"	1 yr	"	++	Not done	
10	GF	3 yr	2 yr	"	++	—	Tubercular synovitis
11	HT	4 mo	3 wk	Prostatitis	—	—	

*Numbers 6 and 9 are female, all others are male. All cultures were negative.

CASE 4—(No 10 on Table I) G F, twenty six years old. Five years before admission he fell down and injured his left knee. Several years ago he became infected with gonorrhea. For two years he suffered with swelling and pain of the left knee joint. There was effusion and a small tumor outside of the joint which was taken for a cyst of the external semilunar cartilage. A synovectomy was done and the membrane was found to be hypertrophic and with necrotic villi in some places. Sections showed numerous tubercles. Twenty cc of fluid was recovered with some red cells. *Gonococcus* fixation was two plus and Wassermann was negative in the fluid, while in the blood they were both negative.

c In 79 fluids cultures were made and 19 were positive for different strains of staphylococci, streptococci, and diphtheroids. None of these fluids gave positive gonococcus reactions.

Check up examinations on reaspiration of a number of fluids gave identical results.

It is therefore concluded that with the eventual exception of a weak positive reaction in one case of tubercular arthritis the presence of specific or nonspecific infection did not interfere with the specificity of the gonococcus complement fixation in synovial fluids.

CLINICAL CONSIDERATIONS

Of the 6 patients with complete gonococcus complement fixation in the synovial fluids, 5 were males between the ages of twenty-three and thirty-six years. All had some urethral discharge. Prostatitis was present in 3 cases and epididymitis in two. The onset of arthritis was from one to eight weeks previous to examination. In 4 cases several joints were involved the knee joint being chiefly affected. One patient suffered from an arthritis of the ankle joint only. The clinical picture was an acute infectious arthritis with

pain peri-articular swelling, limitation of motion and effusion. The synovial fluid was turbid with a high cell count and contained polymorphonuclear leucocytes from 60 per cent to 90 per cent. In 4 of these cases, the gonococcus complement-fixation test in blood serum was carried out and gave a four-plus reaction. The cultures were negative in all cases for gonorrhea and other organisms. In one case gonococci were demonstrated in the smear from the sediment of the synovial fluid. Of the 5 cases with partial complement fixation one gave a three-plus in the synovial fluid while the blood serum was negative.

CASE 5—(No. 7 on Table I) M. A., twenty-seven years old. He had gonorrheal urethritis two and one half years previously and an arthritis of both knees two years ago. For three weeks he had had effusion in both knees and transitory pains and swelling in feet, ankles, and right sternoclavicular joint. The fluid aspirated from the knee joint was cloudy and contained 92 per cent polymorphonuclear leucocytes, the cultures were sterile after eight weeks. There was no involvement of the heart, no evidence of still persisting gonorrheal infection. Salicylates had little effect and improvement followed after injection of the filtrate from the synovial fluid.

Of the three patients who gave a two-plus gonococcus complement fixation in the fluids, one (Case 4) was proved to be tubercular arthritis of the right knee. The second patient a man thirty-eight years old suffered from a subacromial bursitis for two weeks previously. The effusion was turbid and contained 75 per cent polymorphonuclear leucocytes. He did not admit gonorrheal infection, no urologic examination was carried out and blood serum was not tested. The third case was a woman of twenty-nine years who gave the history of an acute polyarthritis three years before. For three weeks she has had a recurrence with pain in the feet and wrists and effusion in the right elbow joint. No record of gonorrheal infection was obtained and blood serum was not tested.

One patient gave a one plus positive complement fixation in the synovial fluid while the blood serum was negative.

CASE 6—(No. 11 on Table I) H. T., thirty-six years old. Four months previously he had a gonorrheal infection. Seven weeks ago there was a sudden onset of pain, swelling and effusion in the right knee, with an increase in local and general temperature. The synovial fluid was cloudy and contained 98 per cent of polymorphonuclear leucocytes. The prostatic gland was swollen and tender. The secretion was purulent. No gonococci were found. An improvement was effected by intravenous injections of typhoid vaccine.

COMPARISON OF GONOCOCCUS COMPLEMENT OF BLOOD SERUM WITH SYNOVIAL FLUID

The complement-fixation test was carried out simultaneously in the blood serum in 8 cases. In 4 cases the reaction was four-plus in agreement with the results in the synovial fluid. These were cases of acute arthritis with other evidence of still persisting gonorrheal infection and complications. In one case of chronic infectious arthritis, the reaction was two plus in the serum while the synovial fluid gave a four-plus. In three cases with partial fixation in the synovial fluid the blood serum was negative. Of these, one patient had acute arthritis of the knee and a urethritis and prostatitis. Two had old gonorrheal infections but no evidence of recent activity. One of these was histologically proved to be a case of tubercular synovitis. The synovial

fluid gave a higher percentage of positive reactions and a stronger reaction than the blood serum. Some evidence can be adduced that this is due to a higher concentration of antibodies in the synovial fluid rather than to interference by unspecific substances. In 95 fluids, including 36 cases of acute inflammatory arthritis the reaction was negative. A number of these cases had a history of gonorrheal infection. The patients with strong positive reactions and at least one with a weak positive reaction gave other evidence of active gonorrheal infection. On this basis we must consider that the partial gonococcus complement fixation in the synovial fluid alone may indicate that gonorrhea is at least one of the factors involved in the etiology of the arthritic condition. However the material is too limited and further studies are needed to definitely estimate the value of partial gonococcus complement fixation in the synovial fluid.

CONCLUSIONS AND SUMMARY

1 The technic of the gonococcus complement-fixation test in the synovial fluid is outlined and the results in 106 cases of acute and chronic inflammatory arthritis are reported.

2 The reaction was found to be positive in 11 cases (10.4 per cent). The presence of other infections does not interfere markedly with the specificity of the reaction.

3 In 7 cases other evidences of active gonorrheal infection were found but in 4 cases this reaction was the only evidence of the gonorrheal etiology.

4 In 4 cases both blood and synovial fluid gave complete gonorrheal complement-fixation tests. In one case the blood gave only a weak positive reaction while the synovial fluid gave complete positive reaction. In 3 cases the synovial fluid gave partial complement fixation and the blood was negative.

5 The strong positive reaction in the synovial fluid is therefore considered a valid proof of the gonorrheal etiology of the arthritis and more conclusive than the reaction in the blood serum where it only indicates the presence of an active focus somewhere in the body.

6 The significance of partial complement fixation in the synovial fluid needs further investigation.

7 It is recommended on the basis of these findings to carry out the gonococcus complement fixation tests as a routine examination in all synovial fluids.

NOTE. The material for this study was derived from the orthopedic services of Drs. Harry Finkelstein, Herman Frauenthal, Samuel Kleinberg and Leon Mayer to whom we express our appreciation.

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THE EFFECTS OF ULTRAVIOLET IRRADIATION ON THE REDUCING POWER OF BLOOD†

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KOCH and Reed,^{1*} irradiating, with a carbon arc lamp blood flowing through a quartz tube inserted into the carotid artery in etherized dogs, reported increased uric acid values. This increase, an average of 38 per cent in twenty-one dogs, was determined by the phosphotungstic acid colorimetric method of Folin and Wu as modified by Koch.^{1*} It was recognized at that time that there was no actual increase of uric acid in the blood, but that the reaction was due to other reducing substances.

The experiment was repeated by Reed and Barnard¹ with somewhat varying results sufficient to justify a reinvestigation to determine, if possible, the interfering substances.

EXPERIMENTAL PROCEDURE

Forty to fifty cc of blood were drawn from a dog, using $\text{Na}_2\text{C}_2\text{O}_4$ as an anticoagulant. This sample was divided into three portions. The first portion was analyzed immediately. The second was placed in a fused quartz flask 5 mm in thickness and irradiated with a Kromayer quartz-mercury vapor lamp at a distance of one inch (at this distance there was uniform diffusion throughout the flask) at room temperature. Samples were analyzed at thirty, sixty, and ninety minute intervals. The third portion from the original sample was placed in a glass flask similar to the one used in irradiating the blood, set in an adjoining room as a control and analyzed after ninety minutes. Analyses were made directly upon the blood filtrates according to Folin's¹⁰ method. The results of this experiment are shown in Table I. An increase in reducing power in irradiated blood is noticeable in only one in-

TABLE I

DOG	ORIGINAL	MINUTES			IRRADIATED	CONTROL
		30	60	90		
I	1.70	2.16		2.20	2.15	1.60
II	1.39	1.34		1.14	1.28	1.42
III	0.66				0.80	0.72
IV	0.75			0.90	0.85	1.00
V	2.81				2.64	
VI	0.97				0.86	
VII	0.86				0.89	
VIII	1.47				1.53	
IX	1.54				1.51	

Results are in mg. uric acid per 100 cc. blood. For obvious reasons some of the samples were not analyzed.

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stance Number K. In this case, however the colorimetric readings were as 16/22 which introduces an error large enough to account for the results. The other experiments show no increase whatever outside of the percentage of error.

In view of the fact that Koch and Reed had used a modification of Folin's uric acid method, it was thought that perhaps this might account for failure to duplicate their results. In the last three experiments VII, VIII and IX, samples were also analyzed by Koch's modification of Folin's method which is essentially the same except that 5 c.c. of 4 per cent NaCN in N/7 lithium hydroxide is substituted for 2 c.c. of 15 per cent NaCN in N/10 NaOH. No increased reduction was noted though there was a difference in color produced. This is indicated in Table II.

Ten c.c. portions of the above samples were added to 7 c.c. of Silver lactate, the mixture centrifuged, the supernatant liquid poured off and the precipitate washed with 10 per cent NaCl in N/10 HCl. This washing gave no color deep enough to read with either set of reagents.

TABLE II

DOG	FOLIN'S		90 MIN. IRRADIATION	
	Koch	Folin	Koch	Folin
VII	12	0.86	113	0.89
VIII	141	1.47	149	1.53
IX	112	1.54	112	1.51

Results in mg. uric acid/100 c.c. blood

As indicated in Tables I and II the results of Koch and Reed *in vivo* cannot be produced *in vitro*. This may be due to a mechanical effect difficult to obviate. In irradiating whole blood the proteins coagulate in a film on the side of the flask nearest the lamp which may prevent penetration. There is also a tendency for the corpuscles to settle out, thus reducing the surface of contact between corpuscles and plasma. In order to determine the importance of this effect the following experiment was made.

Uric acid in the presence of ultraviolet light loses its power to reduce phosphotungstic acid as indicated.

TABLE III

SOLUTION	AFTER 90 MIN. IRRADIATION	PER CENT LOSS
1 92.5	90.6	2.3
2 19.25	11.9	38.2
3 0.96	0.179	81.3

Mg. uric acid/100 c.c.

Solution 1 dissolved in Na_2CO_3

Solution 2 and 3 dissolved in Li_2CO_3

Exactly 10 mg. of uric acid (Folin Standard) was added to 40 c.c. of dog blood and thoroughly mixed. A sample was analyzed immediately and the remainder was divided into two portions, one of which was irradiated ninety minutes, the other was used as a control. Proteins were precipitated with tungstic acid and 10 c.c. portions of the filtrate were analyzed for uric acid by Folin's isolation procedure.¹⁰

TABLE IV

ADDED	RECOVERED	AFTER IRRADIATING 90 MIN	CONTROL
2.5	1.61	0.38	1.57
2.5	1.18	0.94	1.25

Mg uric acid/100 c.c.

In both cases a film was formed on the side of the flask and the loss of uric acid corresponds roughly to the penetration. The amount of recovered uric acid is about the same as reported for human blood and for sheep blood.^{4, 11}

The newer method of Folm,¹¹ however, recovers uric acid quantitatively from sheep or human blood. We may add that the method works as well for dog blood.

The above experiment was repeated using this newer method of Folm on unclotted blood extract on the only blood available at the time from a pregnant bitch. In this case, however, of the 4 mg per cent added the same amount was recovered immediately, ninety minutes later, and after another ninety minutes interval and also after ninety minutes' irradiation. The film on the flask came off as a coating.

To determine the relation of glucose aqueous solutions of glucose (Merck's cp dried twenty four hours at 85°) were irradiated under conditions as given above. After irradiation the samples were analyzed with Folm uric acid reagents¹⁰ and matched against a standard of uric acid. In using this standard interference of glucose in blood uric acid determinations can be seen at a glance, but, more important, uric acid presented a constant standard for determining a change in reducing power of the glucose solution.

The results of Table V seem to indicate the inconstancy of this method while dilute solutions of substances other than uric acid the variation being inversely as the color produced. Similar variations are shown in Tables I and II.

TABLE V

GLUCOSE PER CENT	MINUTES IRRADIATED	ANALYSIS (1) MG URIC ACID PER 100 C.C.	ANALYSIS (2)	ANALYSIS (3)	CREATININE DIFF.	PHENOL DIFF.
2.00	0	1.10	0.90		0.20	20
1.75	0	0.77	0.98		0.21	25
1.50	0	0.49	0.56	0.42	0.14	28
	30	0.54				
	60	0.57				
	90	0.45	0.54	0.62	0.17	31
1.25	0	0.49	0.52	0.40	0.12	25
	30	0.58				
	60	0.58				
	90	0.54				
1.00	0	0.29	0.47, 0.29	0.38	0.18	36
	30	0.45	0.51		0.03	3
	60	0.45				
0.8		0.31				
0.6		0.20				
0.4		0.16				
0.1		0.05				
		0.05				

The interference of glucose in the analysis of normal blood filtrates for uric acid is practically none even in cases of diabetes with a blood sugar of 1 per cent the interference would be less than 0.5 mg/100 cc of blood if a 'direct' method was used. This interference is practically nil in view of the increased uric acid in diabetic patients.

DISCUSSION

It is important to note that the so-called uric acid value by no means represents uric acid. It is to be exact the value obtained by reducing phosphotungstic or arsenophosphotungstic acid in the presence of alkali and cyanide compared with a standard of uric acid. The color produced obeys Beer's Law only within very narrow limits, so the blood filtrate and the standard should be very nearly the same concentration or sizable errors are introduced in finally matching the colors. A number of substances also reduce the reagent or otherwise influence the reaction. Grigaut¹⁷ after trying some hundred compounds that may be present in protein-free blood filtrates, reports that uric acid, alloxane, and alloxantine alone reduce phosphotungstic acid but polyphenol medicants influence the reaction. Incidentally this observer finds that glucose does not influence the reaction.

The findings of Grigaut could not explain the discrepancies noted by workers in this country between the values given by "direct" and "indirect" uric acid methods.¹⁸⁻¹⁵ These discrepancies indicated the presence of a substance, or substances that interfered with uric acid determination when made directly upon the protein-free blood filtrates. Especially was this true of animal bloods in which the actual uric acid content is thought to be 0.1 mg per 100 cc or even less. As a result of this Benedict, Newton and Behr³ isolated a compound from pig blood which they called thiasine. Independently and at about the same time Hunter and Eagles¹² isolated a compound from pig blood having very similar properties, which they called sympectothion. It was later shown by Newton, Benedict and Dakin¹¹ that thiasine was identical with ergothionine isolated from ergot of rye and with the sympectothion of Hunter and Eagles. For this compound, the betaine thiolhistidine, Benedict proposes the name thionine for the one of animal origin. The work of Newton, Benedict and Dakin was verified independently by Eagles and Johnson.¹⁶

When Blumer, Eagles and Hunter⁷ were isolating Substance X or sympectothion or thionine they suspected the presence of another substance which also reduced phosphotungstic acid. They later isolated this substance which proved to be glutathione.¹⁶

Thus within the last few years two unsuspected substances have been isolated from blood which influence 'direct' uric acid determination and it is indicated that there are others. Rockwood, Turner and Pfaffner²⁰ report the presence of another interfering substance after acid hydrolysis of tungstic acid blood and tissue filtrates. They call this Substance Z. Behr and Benedict³ in determining the thionine content of human blood conclude that there must be one other substance (other than uric acid, thionine or glutathione)

which yields color with uric acid reagents. When viewed in this light the findings of Koch and Reed take on a new significance.

The results of Koch and Reed *in vivo* cannot be produced *in vitro*, the reason is partly, but not wholly, mechanical. Uric acid disappears rather rapidly when irradiated in mild alkaline solution and analyses seem to indicate that it disappears from blood on irradiation provided there is penetration.

No attempt is made to determine the relation of glutathione or thionine to this reaction because methods for analyzing these substances are not well enough established nor could we, at present, observe them in pure solution.

CONCLUSIONS

1 The increase of reducing power of blood irradiated *in vivo* cannot be duplicated by irradiation *in vitro*.

2 Uric acid under the influence of ultraviolet irradiation loses its power to reduce phosphotungstic acid.

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THE RELATION OF THE P_H REACTION OF URINE TO THE ANTISEPTIC ACTION OF MALLOPHONE IN VITRO*

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SEVERAL urinary antiseptics of the pyridine type have been placed upon the market during the last few years. These drugs are administered orally and are known to be excreted selectively by the kidneys so that practically all of the ingested drug is eliminated in the urine. We have studied one of these drugs known as mallophone (beta-phenyl-azo-alpha-alpha-diamino-pyridine hydrochloride) quite extensively during the last several months. Clinical observations have been made, but more particular attention has been given experimentally to the relation of the hydrogen-ion concentration of the urine to the bactericidal and bacteriostatic action against staphylococci and colon bacilli in the presence of various dilutions of mallophone.

Mallophone has many of the characteristics of an ideal antiseptic since it seldom produces any disturbance of the intestinal tract, and can be given in therapeutic dosage over a long period of time without any apparent irritation of the kidneys. Such a dye compound is perhaps more penetrating than many other antiseptics. While the amount excreted in the urine is not completely bactericidal for the colon bacillus, it is sufficiently bacteriostatic that when used over a fairly long period of time it seems to produce bacteriologic cure in many of the acute uncomplicated types of infection. When combined with pelvic lavage and drainage in the chronic type of infection it has given better results clinically in my experience than any other urinary antiseptic previously used. In the typical acute case it is advisable to give one tablet three times a day for a few days to determine tolerance for the drug, and then the dosage is increased to two tablets two or three times a day until the amount of pus and number of bacteria in the urine are definitely decreased. Later the dosage is decreased to two tablets once daily until sterile cultures have been obtained. It is advisable to continue two tablets every other day for a short period after bacteriologic cure has been obtained to decrease later exacerbations.

A few clinical papers have been published during the last several years in which stress has been made of the importance of acidity as a factor in the successful management of urinary infections but its necessity is more generally considered to be a means whereby formalin is liberated from hexamethylenamine. More recently Hiller and Stamler¹ have noted that mercurochrome is more bactericidal in acid than in alkaline mediums by laboratory tests. There was an exception in a short range on the acid side.

Preliminary observations were made by the addition of varying quantities of 2 per cent acid sodium phosphite and 2 per cent alkaline sodium phosphate to the same specimen of urine. Dilutions of mallophone were then added and

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inoculations made with colon bacilli. Subcultures were made after three days on plain nutrient agar. The results are tabulated in Table I. Hydrogen-ion concentrations were not made in this preliminary observation, but the antiseptic action of mallophone was greater in the urine specimens containing the acid sodium phosphate. There was a slight inhibition of the growth of the colon bacilli in the urine specimen containing acid sodium phosphate alone as compared to the urine with alkaline sodium phosphate alone.

Further observations were then made to determine the comparative bactericidal action at known hydrogen-ion concentrations. Urine was used as the medium for addition of various dilutions of mallophone since it seemed logical that such observations would be more comparable to later experiments *in vivo*. The technique served as a means for the determination of the bacteriostatic action as emphasized in the "growth-restraining" test of Leonard² since urine serves as a good medium for the growth of colon bacilli and staphylococci. In addition the transfer test at the end of three or four days' incubation gave a consistent bactericidal result for comparative purposes. The results tabulated in Table II

TABLE I

IN VITRO CULTURAL RESULTS OF B. COLI ADDED TO ACID OR ALKALINE SODIUM PHOSPHATE IN URINE WITH MALLOPHONE

DILUTION OF MALLOPHONE	90 CC URINE 88.5 CC 2% ACID SODIUM PHOSPHATE	90 CC URINE 88.5 CC 2% ACID SODIUM PHOSPHATE	90 CC URINE 88.5 CC 2% ALKALINE SODIUM PHOSPHATE	90 CC URINE 88.5 CC 2% ALKALINE SODIUM PHOSPHATE
1-2000	0	0	3+	3+
1-4000	0	0	4+	4+
1-8000	0	0	4+	4+
Control urine without mallophone	3+	3+	4+	4+

indicate that there is a very definite increase in the bactericidal action for colon bacilli and staphylococci at the acid range of P_H 5.2 and P_H 5.8 as compared to acid range P_H 6.6 and alkaline range P_H 7.4. In this experiment a single urine was used and the specimens voided during the same day starting with the acid P_H 5.2. Then alkalies were taken by mouth, and succeeding specimens were collected to obtain urines with the higher hydrogen-ion concentration as indicated in Table II so that the difference in hydrogen-ion concentration was the only variation in the medium with the same mallophone dilution. If these results prove consistent with similar observations *in vitro* it would seem worth while to consider the routine hydrogen-ion determination as a means of obtaining the maximum efficiency of urinary antiseptics in the management of urinary

TABLE II
MALLOPHONE ADDED TO URINE IN VITRO

P_H	COLI				STAPHYLOCOCCI			
	1000	2000	4000	6000	1000	2000	4000	6000
5.2	0	0	0	2+	0	0	0	1+
5.8	0	0	0	3+	0	0	0	2+
6.6	3+	3+	4+	4+	0	2+	3+	3+
7.4	4+	4+	4+	4+	2+	2+	3+	4+

infections. We have noted that the range of hydrogen-ion concentration may be estimated roughly by boiling a specimen of urine for five minutes in a test tube. If clouding occurs after heat the P_H is higher than 6.0 and antiseptics in such urine are of less value, but if the urine remains clear the acidity is below P_H 6.0 and within or bordering on the optimum range.

It was interesting to note the average hydrogen-ion concentration of the urines in a series of 75 patients. These specimens were tested immediately after voiding, and without the influence of urinary antiseptics or urinary acidifying agents. The results are tabulated in Table III and are divided into two classes, private and clinic patients. It may be noted without any definite significance that a higher percentage of private patients in this small series of cases had urines with a hydrogen-ion concentration within the range that we would not consider to be the optimum for urinary antiseptics, particularly mallophenc, as indicated by the above bactericidal tests. If this comparative increase in the higher hydrogen-ion concentration with private patients should prove consistent with a larger series of cases, it might be explained because of the difference in time of observation. The private patients were seen in the afternoon or evening while most of the clinic patients were seen during the morning hours. Again there may be some variation as the result of the dietary differences of the two classes of patients.

TABLE III
ROUTINE P_H OF PATIENTS ON EXAMINATION

TYPE OF PATIENTS	NUMBER WITH P_H 4.8—5.2 INCLUSIVE	NUMBER WITH P_H 5.3—5.7 INCLUSIVE	NUMBER WITH P_H 5.8—6.2 INCLUSIVE	NUMBER WITH P_H 6.3—6.7 INCLUSIVE	NUMBER WITH P_H 6.8—7.2 INCLUSIVE
Clinic	20	16	6	4	1
Private	7	5	7	4	5

DISCUSSION

It is evident that if the hydrogen-ion concentration influences the optimum range of action of urinary antiseptics *in vivo* as it clearly does in experiments *in vitro*, more importance should be given to such observations in urinary infections, and particularly to the time relationship between the administration of the urinary antiseptic and the urinary acidifier. Fiske² states that an alkaline tide occurs regularly after meals. We have made comparative observations of three commonly used acidifying drugs: acid sodium phosphate, sodium benzoate, and ammonium chloride. While the observations have not been sufficiently extensive to draw definite conclusions, we have noted that ammonium chloride is less likely to be followed by a temporary change toward the alkaline range than is acid sodium phosphate and sodium benzoate. In one instance a dose of four grams of acid sodium phosphate was given and a specimen two hours later had a hydrogen ion reading of 5.5. Specimens taken at intervals up to six hours indicated a gradual increase in the hydrogen ion reading so that six hours after the ingestion of acid sodium phosphate the P_H reading was 6.1. It would seem that further observations are necessary on the influence of the size of the dose of the various acidifying drugs.

The impression is gained by the observation of repeated hydrogen-ion determinations at intervals of several days that certain individuals are likely to have regularly, a reaction consistently low on the acid side. Others seem more likely to have reactions consistently high on the acid side and even occasionally alkaline.

CONCLUSIONS

1 Contrary to the usual belief, antiseptics particularly of the pyridine type seem to be more efficient by experiments *in vitro* when added to urine in the lower acid range of hydrogen ion concentration than in the higher acid or alkaline range.

2 The routine hydrogen-ion determination in a series of 75 patients indicates that a majority are within the limits of optimum reaction for efficient antiseptic, yet there is a sufficient number in the less efficient range to indicate a necessity for acidifying agents to obtain the greatest efficiency of the urinary antiseptics.

3 Thus increased efficiency of mallophone in the more acid urines seems to apply equally to colon bacilli and staphylococci as indicated by experiments *in vitro*.

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LABORATORY METHODS

THE ESTIMATION OF THE SERUM CAROTIN*

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THE yellow pigments designated as lipochromes or carotinoids have been the subjects of many investigations. A very complete bibliography up to 1922 is contained in the monograph by Palmer¹ while more recent work is reviewed in the papers by Boeck and Yater² and by Stannus.³ Our present knowledge of these pigments in connection with human blood serum can be summarized briefly. The pigments carotin and xanthophyll are present in small quantities in the blood of normal individuals, giving a yellow color to the serum, and can be estimated by means of a technic elaborated by Palmer. Under certain conditions, notably in cases of diabetes, the amount of this pigmentation is greatly increased and it has been shown that this increase is chiefly due to increased quantities of carotin, hence the condition is known as carotinemia although the more general term, xanthemia, is also used. If the xanthemia is very pronounced it may be accompanied by skin pigmentation or xanthosis sometimes referred to as pseudoicterus since it resembles the pigmented appearance of jaundice but differs therefrom in that the sclerotics are not involved. The condition of xanthosis has also been observed in nondiabetic individuals, chiefly children on a diet rich in carrots. Elimination of this vegetable from the diet generally leads to restoration of the normal skin coloration.

As far as is known at present, an increase in the carotin content of the blood has no particular clinical significance, and can be controlled easily by adjustment of the diet. Its possible importance however, cannot be ignored since we are also in ignorance of the function normally fulfilled in the organism by the carotinoids. The last decade has added considerably to our knowledge of carotin: the close association in plants and vegetables between lipochrome pigments and the fat-soluble accessory food factor vitamin A has led to the discovery that minute amounts of purified crystalline carotin (but not xanthophyll) when added to vitamin A-free diets produce effects comparable in every way with those of vitamin A^{4, 5} and that in effect, carotin can now be regarded as the precursor of that vitamin in the animal organism.⁶⁻⁸ Through the researches of Willstätter and Stoll, van den Bergh and his coworkers,¹⁰⁻¹¹ Palmer and others, methods are now available for detecting the presence of carotin in serum and estimating it quantitatively. These

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methods primarily depend upon the solubility of carotin in low boiling petroleum ether and the fact that this solvent will remove carotin quantitatively from its solution in 80 to 90 per cent alcohol. The latter fact is of considerable importance since Palmer has shown that the pigment is apparently in some form of combination with the serum protein which prevents its direct extraction. Treatment of the serum with alcohol precipitates the proteins and releases the pigment, which can then be extracted. As all other pigments likely to be present, including hemoglobin and bilirubin, remain dissolved in the alcohol the petroleum ether extract can be removed and compared against a standard solution. This in broad outline is the procedure which is universally adopted, only variations in the details of the technic distinguishing the methods of different investigators.

Since solutions of pure carotin are unstable for routine determinations an artificial standard is essential and it has been found that a dilute solution of potassium dichromate gives a color sufficiently close to that of carotin to enable it to be used instead. A perusal of the literature reveals that in the strength of the dichromate standard solution used and its color value in terms of carotin lie the greatest divergences between the various methods of estimation. The chief difficulty, which has not always been appreciated, is that the relationship between concentration and color intensity in an aqueous solution of potassium dichromate is not the same as exists in a petroleum ether solution of carotin. This is exemplified in Willstatter and Stoll's use of a 0.2 per cent solution of potassium dichromate as standard. They found that a 5×10^{-4} molar solution of carotin (0.00268 per cent) compared in a colorimeter at a depth of 100 mm against this dichromate solution gave a color match when the depth of field of the latter was 101 mm. When the carotin solution was placed at 50 and 25 mm respectively, a color match was obtained with the dichromate solution at the respective depths of 41 and 19 mm. Notwithstanding these divergences of color intensity at different depths of field, colorimetric comparison without adequate corrections has been consistently used in evaluating the degree of carotinemias, and consequently where the values are expressed as actual carotin content there are inevitably serious discrepancies.

If the depth of field be kept constant then this error is avoided, together with the not inconsiderable error due to the comparison of a very volatile liquid with a nonvolatile one in open colorimeter cups. The following procedure was therefore worked out with the idea of obtaining a routine laboratory method which could be used with a reasonable degree of accuracy. The manipulative procedure is practically that of Palmer as used by Rabinowitch¹² with small amounts of serum but adapted to give values which can be expressed in terms of carotin. It consists in extracting a mixture of serum, plaster of Paris and alcohol, with petroleum ether, transferring the extract to a clean dry test tube and comparing it against a set of previously prepared standards. As it is in the choice of suitable standards and their carotin value that the utility of the method depends, it is necessary to deal with this in some detail.

The Selection of Standard Solutions—

When this investigation was commenced Rabinowitch's method was followed, utilizing as standards petroleum ether solutions of oleic acid but these were found to be unsatisfactory. Oleic acid is an oily liquid at ordinary temperatures and when chemically pure is colorless. Being an unsaturated compound it readily oxidizes on exposure to air and the greater the amount of impurity present the greater is the color which it possesses. Rabinowitch himself admits that his standard solutions deteriorate and should be renewed weekly, for a permanent standard he recommends the potassium dichromate solution adopted by Connor.¹³ Another objection to the use of oleic acid is the difficulty of obtaining uniformity of coloration when the acid is obtained from different sources. We compared a number of samples of different grades of oleic acid which were in stock in each case making up a 50 per cent solution in petroleum ether. Taking the purest sample as equivalent to 5 units of pigment (*vide* Rabinowitch), four samples had values of 5, 11, 20 and 30 units respectively whilst two very impure samples were so strongly colored as to be noncomparable. It was accordingly decided to use potassium dichromate as the standard. A 0.2 per cent solution was made up and taken empirically as 100 units and a series of test tubes (each of monax glass and 13 mm bore) prepared, containing the dichromate solution suitably diluted to represent 1 to 100 units. These test tubes sealed and kept out of contact with light, contained the series of standards. It still remains to determine their carotin equivalent. No pure carotin being available at that time a carrot was extracted with petroleum ether, and without further manipulation the strongly colored extract was diluted with petroleum ether until it gave almost exact color comparison with the 0.2 per cent dichromate solution both solutions being compared in test tubes of 13 mm bore using a simple color comparator. This extract was then progressively diluted with the solvent until a series of dilutions were obtained similar to those of the dichromate. On comparing these diluted carotin solutions with the corresponding dichromate solutions it was observed that as noted by Connor progressive dilutions of the two solutions did not give corresponding values. The values were therefore plotted against one another and were seen to lie approximately on two straight lines which intersected at a point equivalent to 47.5 dichromate units. To verify this unexpected finding on two other occasions petroleum ether extracts of carrots were made, progressively diluted compared with the dichromate standards and the results plotted. Although the carotin content of these extracts varied in each case a graph similar to the first one was obtained with the point of intersection having the same dichromate value 47.5 units. Further the results from all three experiments when plotted together could be represented by one pair of intersecting straight lines. In order to relate this to actual carotin content a sample of crystalline carotin was prepared from carrots and recrystallized. It had a melting point of 167°-168° C (uncorr.). A solution containing 4.0 mg. of the crystals in 100 c.c. was found to give color comparison with 111 dichromate units (100 units being represented by a 0.2 per cent solution). Fig. 1 therefore represents the values

expressed in mg carotin per 100 cc of a 0.2 per cent solution of potassium dichromate and its progressive dilutions

The reason for the sudden change in the angle of inclination of the graph is unknown. It is not a property of the dichromate solution, this has been tested and found to give a straight line when the color intensity is plotted against the concentration throughout the whole range. The explanation may lie in the fact that the petroleum ether used (Merck's c.p. B.P. 30°-80° C) was a mixture and not a true chemical compound, but in any case it is only of theoretical interest, since our experience has shown that even most pronounced cases of carotinemia have values which lie in the lower segment of the graph. This part of the graph can be represented by the equation

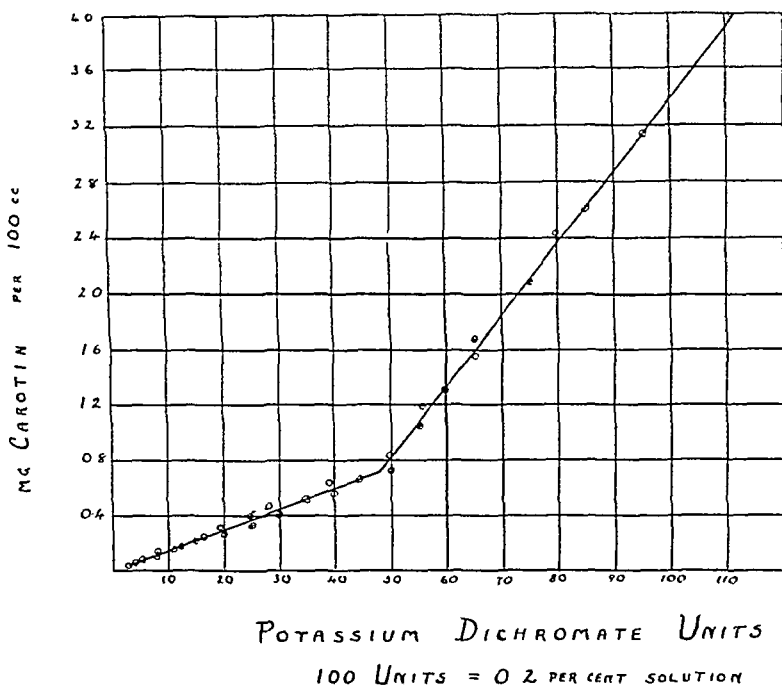


Fig 1—Graph showing the carotin values of potassium dichromate units at a depth of 13 mm

$x = 0.375 y$ (where y = number of dichromate units and x = number of carotin units), and as each carotin unit represents 0.04 mg of carotin in 100 cc of petroleum ether the equation can be further simplified and written thus $X = 0.015 y$ (where X = mg carotin per 100 cc)

As previously mentioned Willstatter and Stoll's results showed that a dichromate solution gave varying values when compared against the same carotin solution at different depths of field. It is not therefore possible to contrast then results with varying concentrations of dichromate and carotin solutions at the same depth of field, such as is now represented graphically. For the same reason Connor's tabulated data of the carotin value of different strengths of dichromate solution at a depth of field of 30 mm, are not comparable with our results which were obtained with a depth of field of 13 mm

Details of the method of estimation are as follows. Two c.c. of serum are pipetted into a graduated centrifuge tube, and to this is added (in small portions) 4 gm. of plaster of Paris the serum being stirred after each addition by means of a thin glass rod. When all has been added the contents of the tube should have the consistency of a thick paste. Two c.c. of 95 per cent alcohol are then added, and after again mixing thoroughly the glass rod is withdrawn, and 2 c.c. of petroleum ether pipetted in. The tube is immediately stoppered with a close fitting cork stopper (rubber is attacked by petroleum ether), shaken either by hand or (preferably) mechanically for ten minutes and then centrifuged. The contents will be found to have separated into 3 layers of which the top contains all the carotin pigment. The top layer is measured and transferred to a clean dry test tube of 13 mm. bore, which is tightly stoppered, and the colored liquid compared against the dichromate standards until a match is obtained. The value in dichromate units being thus obtained, from the equation the equivalent value in terms of carotin is calculated. As this is expressed in mg. per 100 c.c. a correction has to be applied to compensate for the solution of a fraction of the petroleum ether in the alcohol layer. This is allowed for by multiplying by a factor, obtained by dividing the measured amount of the petroleum ether layer by the volume of petroleum ether taken. After some practice we found that the petroleum ether layer could be taken as 1.8 c.c., giving a factor of 0.9. In Table I is shown a summary of results obtained with this procedure.

TABLE I

NO. OF CASES	DESCRIPTION	MG. CAROTIN PER 100 CC. SERUM	
		MEAN	EXTREMES
18	Normal	0.063	0.024 - 0.108
14	Diabetic	0.213	0.072 - 0.379
8	Pathologic but nondiabetic	0.055	0.032 - 0.092

The Influence of the Serum Carotin upon the Icterus Index—The icterus index introduced by Meulengracht¹⁴ as a measure of the degree of bile pigmentation of serum consists simply in matching the color of the serum against a standard solution of potassium dichromate and is largely used as a routine test in cases of jaundice. In such cases it is assumed that the amount of carotinoid pigment present is not markedly increased and that consequently the index gives a fairly reliable indication of the amount of bilirubin present in excess of normal. As amended by Maue¹⁵ the procedure consists in comparing in the colorimeter a suitably diluted serum against the standard, the icterus index being calculated from the formula

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} = \text{dilution}$$

It follows that when the standard and unknown readings are equal the icterus index is equal to the dilution. The standard used is a 0.01 per cent solution of potassium dichromate so that if a 0.2 per cent solution is represented by 100 units then the more dilute solution represents 5 units which is equivalent to 0.075 mg. carotin per 100 c.c. The highest value which we obtained for

nondiabetic serum was 0.108 mg carotin per 100 cc so that the color due to carotin in this serum could be represented by an icterus index of 1.4. The normal range for the icterus index according to Bernheim¹¹ is 4 to 6 and these limits are generally accepted, consequently the color due to carotin in the sera of nondiabetic individuals apparently can have but little influence upon the icterus index. With cases of diabetes however the carotin content increases considerably. Of the 14 cases reported in Table I the highest value obtained was 0.379 mg equivalent to about 25 dichromate units. This is five times the strength of the icterus index standard and can therefore be represented by an index of 5. This superimposed upon the normal value brings the index within the zone of latent jaundice. On the other hand such a value would be accompanied by xanthosis and other symptoms which could be recognized clinically. It can be concluded therefore that except in cases of well-marked carotinemia such as sometimes accompany diabetes, the color of the serum due to carotin has very little effect upon the icterus index.

SUMMARY

Details are given of a modification suitable for routine determinations of Palmer's method for the estimation of serum carotin. It can be carried out on 2 cc of serum and the results expressed with reasonable accuracy in terms of mg carotin per 100 cc serum.

It is concluded that except in cases of marked xanthemia accompanying diabetes the carotinoid pigmentation of the serum does not influence the icterus index to any appreciable extent.

We wish to express our indebtedness to Dr. C. Hunter, on whose suggestion this investigation was undertaken, and in addition, to Drs. F. J. Hart and N. W. Warner, for permission to investigate their cases and for their interest and cooperation. We also wish to thank Professor A. T. Cameron for his criticism and advice.

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THE EFFECT OF AMYL NITRITE UPON THE FINGER VOLUME*

BY CARL A JOHNSON, M D CHICAGO III

THE prevalent opinion as to the action of amyl nitrite upon the peripheral vascular system is that of a generalized vaso dilatation the effect being more pronounced in the face and neck region than elsewhere

Accepting this view we wished to use this drug as a control drug in a study of the factors modifying finger volume Our results were rather striking and hence we felt justified in making this brief report

PROCEDURE

Many plethysmographs have been described, but for the particular purpose at hand we used a specially constructed instrument illustrated in Fig 1

It consists of 1 cc pipette graduated to 0.01 cc fused to a one inch test tube The test tube is cut off to any desired length and a glass stopcock fused to the side The open end of the test tube is covered with a rubber dental dam with a hole sufficient to admit a finger snugly

A drop of alcohol containing some pigment such as ink is allowed to run to the center of the pipette The glass stopcock is kept open one finger is put over the open end of the pipette the finger to be tested is inserted into the plethysmograph and the individual takes a comfortable position The glass stopcock is then closed

With the average individual the deflection with each heart beat is approximately 0.01 to 0.02 cc (2 to 3 mm) while with some cases of aortic regurgitation the deflection may be as great as 0.05 to 0.06 cc with each heart beat

Time is allowed for an equilibrium to be reached within the tube for vapor and temperature changes will modify the readings This usually takes about fifteen minutes After a suitable control period, amyl nitrite was given by inhalation and readings taken Normal healthy individuals were used throughout the work and in the sitting posture

RESULTS

The results are given in Table I From this it is seen that for some individuals the finger volume decreases immediately following amyl nitrite amounting to about 0.20 or 0.25 cc It will be noted that in one case (M)

*From the Department of Medicine of Northwestern University and St Luke's Hospital Chicago, Illinois

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TABLE I

THIS TABLE ILLUSTRATES THE EFFECT OF AMYL NITRITE BY INHALATION UPON FIVE NORMAL INDIVIDUALS THE READINGS ARE IN 0.01 C.C.

3/11/31			3/14/31			3/14/31			3/14/31			3/16/31		
JOHNSON			FENN			BISSELL			YOUNG			MILLER		
TIME	READ	RATE	TIME	READ	RATE	TIME	READ	RATE	TIME	READ	RATE	TIME	READ	RATE
3 12	0.4		11 45	0.3		12 57	0.26	92	1 34	0.32	66	10 15	0.3	
3 17	0.39		11 47	0.3	80	12 59	0.26	88	1 36	0.4		10 17	0.28	
3 20	0.41		11 49	0.2		1 00	0.24		1 38	0.49	66	10 18	0.3	
Amyl nitrite			11 50	0.19	76	1 01	0.23		1 39	0.45		10 19	0.31	
3 20½	0.19		11 51	0.23		Amyl nitrite			Amyl nitrite			10 20	0.3	
3 21	0.21		Amyl nitrite			1 02	0.43	136	1 40	0.34		Amyl nitrite		
3 22	0.24		11 51½	0.01		1 03	0.29	84	1 41	0.57		10 20½	0.09	
3 23	0.28		11 53	0.00		1 04	0.14		1 43	0.43	66	10 21	0.11	
3 24	0.30		11 54	0.03		1 05	0.13		1 44	0.43		10 22	0.12	
3 25	0.33		11 55	0.04		1 06	0.13		1 45	0.49		Fainted		
3 26	0.37		11 56	0.2		1 07	0.14		1 46	0.48				
			11 58	0.22										
			11 59	0.32										
			12 01	0.25										
Coughed so			12 02	0.35	76									
experiment			12 03	0.41										
discontinued			12 04	0.5										

the patient fainted. A decreased pulse rate was sometimes associated with the decreased finger volume. Following this the rate and finger volume usually increased above the control value. In one case the total change was 0.53 c.c. (Table I, Dr. F.)

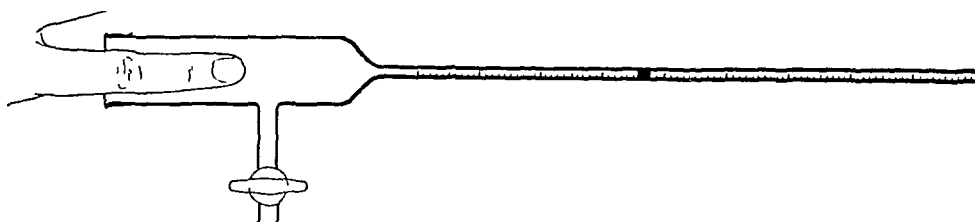


Fig 1

In contrast to the above series of events some individuals show the reverse, i.e., primary increase in finger volume with a subsequent decrease of finger volume below the control value. (Table I, Dr. B.)

DISCUSSION

We have assumed without direct proof that sudden changes in the finger volume are due, in most part, to changes in the amount of blood in the finger vessels. We realize that slower changes in the readings may in part be due to temperature changes in the finger associated with changes in blood supply.

On this assumption we feel that the decreased finger volume in some individuals following amyl nitrite is due to the overwhelming splanchnic dilatation, decreased heart rate and amplitude with consequent fall in blood pressure. This causes a drainage of peripheral blood into the splanchnic region in spite of a possible definite relaxation of the vessels of the finger in question. In other words, blood pressure is insufficient to distend the vessels.

SUMMARY AND CONCLUSIONS

The author has attempted to show by means of a special finger plethysmograph that in some individuals a primary decreased finger volume occurs following amyl nitrite and attributes this finding to the drainage of peripheral blood into the splanchnic region. He is aware of the possibility that definite relaxation of the finger vessels may occur, but the blood pressure is insufficient to distend them.

THE VALUE AND LIMITATIONS OF THE ASCHHEIM-ZONDEK PREGNANCY TEST*

BY ROBERT T. FRANK, M.D., MORRIS A. GOLDBERGER, M.D., AND
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NO SATISFACTORY test for the recognition of early pregnancy was available until the Aschheim-Zondek test appeared in 1928.¹ This reaction is based upon an increase of the anterior pituitary lobe maturity factor in the urine of the pregnant woman. The discoverers of the test have been able to recognize pregnancy as early as three to four days after the expected period failed to occur.

A number of articles confirming the reports of Aschheim and Zondek have been published since 1928.²⁻³ None of them have contained modifications which have proved of value.

The Aschheim-Zondek test in the hands of most investigators and clinicians who have reported on it shows an accuracy of 95 to 98 per cent which, for a biologic test, is extremely satisfactory. Biouha,⁴ however, obtained a positive reaction in only 60 per cent and Mazer and Hoffman in 75 per cent.

Our own experience covering some 350 tests, has proved satisfactory. Certain technical details, sources of error or difficulties, as well as comparisons with other methods of diagnosis, appear worthy of record.

The Aschheim-Zondek test was performed on the urines of 305 patients. Of these 27 cases must be discarded because of the death of all the mice, in a few instances also on repetition. These were all among the first tests performed. The test should be repeated if only one mouse survives and its ovaries are found negative. By using only catheterized urine or where this could not be obtained by first passing the urine through a Berkefeld filter the mortality of the mice has been reduced to a negligible figure.

At various times attempts at varying the technic were essayed. One of the recommended methods was the use of but two mice each receiving 0.5 cc. of urine for 6 doses. Trial showed that the results were less reliable.

Our standard technic differs in only one respect from that of the originators of the test, namely in the employment of four female immature mice

*From the Gynecological Service and Division of Laboratories of the Mount Sinai Hospital.
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instead of five. The mice are injected subcutaneously, respectively with 0.2 cc, 0.25 cc, 0.35 cc, and 0.4 cc at each injection which are spaced two on the first, three on the second and one on the third day. On the fourth day (100 hours after the first injection) the mice are killed with illuminating gas and at once autopsied.

Inspection of the ovaries in positive cases shows one or more minute purplish black spots in the ovaries (anterior pituitary reaction II, APR II) or less characteristic grayish yellow elevations (APR III) resulting from the luteinization of unruptured follicles. APR III can be confirmed as recommended by Aschheim and Zondek, by covering the ovaries with a drop of glycerin and flattening them between a glass slide and heavy cover glass. The luteinized follicles or corpora lutea can then be distinguished by their more opaque and granular appearance, from primordial or maturing graafian follicles if at once examined under the low power of the microscope. This difference in opacity disappears within fifteen minutes as the glycerin "clears" the tissues. In rare instances, if this crude but rapid method proves indecisive serial sections of the ovaries must be cut.

Opening of the vagina as well as enlargement of the uterus and maturation of follicles (APR I) although due to the presence of hypophyseal hormone signifies absence of pregnancy.

In the 321 tests which were completed, 10 errors or 3.1 per cent occurred, all in the direction of negative results in the presence of pregnancy as demonstrated by operation, follow up, or repetition of the test at a later time. In no case was the test positive in the absence of pregnancy. We now regularly crush and examine all macroscopically negative ovaries as, in a not inconsiderable number of instances corpora lutea indistinguishable to the naked eye or to the loupe are thus recognized.

In several instances, especially in the first weeks of pregnancy a negative reaction was noted, followed in a few weeks, on repetition, by a positive result.

Death of fetus did not affect the result as long as the products of conception were retained in utero even for weeks or months. This is in sharp contrast with the blood female sex hormone test⁶ which, as a rule, at once becomes negative. The sole exception to these observations was a case of "missed abortion" with detachment and retention of the ovum, in which the Aschheim-Zondek test was also negative.

Of eleven ectopic gestations, eight gave a positive reaction. One negative reaction was noted in a urine obtained two days after operation, another was accounted for by the complete degeneration of the ectopic ovum. Consequently but one negative result can be ascribed directly to failure of the reaction.

Mazer and Hoffman⁷ described a urine reaction in which 10 cc of suspect urine is injected in 5 divided doses into mature castrates. In only 75 per cent of pregnancies was a positive reaction obtained. In 4 per cent of nonpregnant urines their test proved positive. This high percentage of error in the presence of pregnancy may be accounted for by the fact that our studies⁸ have shown that during the first eight weeks of pregnancy a nega-

tive phase in the production of female sex hormone takes place. The false positives, on the other hand, are due to excess excretion of female sex hormone noted by us⁹ and others¹⁰ in amenorrhea and in various disturbances of the cycle at the approach of the menopause.

The Siddall test,¹¹ which is based on the combined contents of female sex hormone and anterior pituitary hormone in the blood serum of pregnant women with the consequent increase in weight of the genital tract of the injected immature mice gave 17 per cent of false positives in Mazel and Hoffman's hands. It should, therefore, not be employed.

Aschheim and Zondek¹ in 1928 observed that 10 to 0.5 c.c. of blood serum of pregnant women gave a positive reaction (APR II or III). Fluhmann¹² noted the same. We have found serum reaction (using 1.5 c.c. 10 c.c., and 0.5 c.c. totals, divided into 6 injections) almost as reliable as the urine test.

In one case of hydatid mole the urine test was positive, as were the blood and urine female sex hormone tests.

In two cases of chorionepithelioma in the male the Aschheim-Zondek test was positive (one primary chorionepithelioma of testis with retroperitoneal metastases, the second multiple chorionepithelioma of the lung, primary site undetermined). These cases will be reported elsewhere in more detail.

TABLE I

CLINICAL DIAGNOSIS	NO OF CASES	TESTS		WPONG	REMARKS
		-	0		
Pregnancy before eighth week	94	72	35*	4**	*Two repeating became - **Two negatives were pregnant
Pregnancy after eighth week	13	11	2	2*	*One of these was - at 6 weeks but 0 at 11 months
Hydatid	2	1	2*	0	*Negative tests performed 6 weeks after emptying uterus
Ectopic gestation	11	8	3*	1	*One negative test from urine 2 days after operation. One old tubal abortion involuted
Pregnancy vs fibroids	9	5	4	0	
Dead vs live fetus	9	6	5	2	Two positives with dead fetus. Two negatives with live fetus
X ray effect on pregnancy	10	10	14	-	Does not show death of fetus until weeks have passed
Amenorrhea vs pregnancy	50	0	55	0	Fully reliable
Metrorrhagia vs abortion	13	7	9	1*	One became negative on repeat
Chorionepithelioma in male	2	2	0	0	*One became positive on repeat
Follicle fluid	6	2*	4	0	*This was in pregnant patients
Pituitary disease	16	0	15	0	
Miscellaneous	13	1	12	0	The one positive was pregnant the others not
All mice died	27	-	-	-	20 tests made in 3 mice died on repeat
Totals	307	127	106	10	2.1 per cent error
(All mice died)		321	20		
Total Tests		351			

SUMMARY

1 The Aschheim-Zondek test if the original technic is followed, is satisfactory

2 Failure of positive result in pregnancy occurred in 31 per cent

3 If the ovaries are negative macroscopically, it is advisable to examine the fresh, crushed ovaries under the microscope

4 No positive reaction, in the absence of pregnancy, was noted in our series

5 Death of fetus may not influence the reaction for weeks The female sex hormone blood reaction is more delicate under these circumstances

6 The Aschheim-Zondek reaction is also reliable in the diagnosis of ectopic gestation

7 Chorionepithelioma in the male produces a positive reaction

8 The blood serum gives an accurate Aschheim-Zondek test Further report on this will appear later

Our experience with the intravenous Friedman¹³ rabbit pregnancy test, which is said to give a reaction in twenty-eight to thirty six hours, is insufficient to permit of comment or judgment

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THE CLINICAL INCIDENCE OF TRYPTOPHANURIA*

BY ARTHUR T BRICE JR., B A PALO ALTO, CALIF

IN THE presence of concentrated sulphuric acid the aldehydes react with the tryptophane groups of any proteins which contain such groups, producing the characteristic lilac or blue color of the Adamkiewicz reaction. B S Walker and F H Sleeper¹ have shown that the Boltz technic is a specific test for tryptophane based on this reaction as a result of the aldehydes present as impurities in all brands of acetic anhydride. The Boltz test has been extensively employed in the analysis of cerebrospinal fluids, and in November, 1930, I reported its application to urine² together with the results of a brief series of normals indicating that tryptophane is not eliminated in quantities detectable by Boltz's test in the urine of the normal male adult in health and on an average diet and a series of 525 tests on specimens from general surgical and medical cases. This series has now been extended to include over 600 tests from cases of nervous and mental diseases accumulated during recent months.

During the course of the application of the test to date the following observations having a technical bearing have been made. Positive specimens left standing at room temperature overnight without a preservative showed in the morning considerable growth of bacteria and were negative by the test. The same specimens preserved with a few drops of chloroform, ether or toluol remained positive. Positive specimens kept at room temperature in stoppered flasks have been preserved by the use of toluol for as long as a month. Mercuric chloride solutions precipitate tryptophane from urine and preserve it against bacterial decomposition. A very distinct improvement in the technic of the Boltz test when employed in urinalysis has been found to consist in cooling the tube containing the test solutions, either in a large beaker of water or under the tap during the addition of the sulphuric acid. This keeps down to a minimum the production of interfering yellow and brown colors, and renders the test much simpler to read with certainty.

Twenty specimens from general surgical and medical cases have been observed and recorded giving positive Boltz tests in the presence of acetone and diacetic acid. Seven specimens from five different cases of diabetes mellitus have been observed and recorded positive for tryptophane by Boltz test in the presence of sugar. The presence of acetone, diacetic acid and sugar even in considerable amounts therefore does not necessarily mask a positive Boltz test. The removal of ammonia by permittit, or of phosphates and carbonates by barium chloride and hydroxide or of carbohydrates by copper sulphate and calcium hydroxide from the average specimen of urine does not appreciably simplify the reading of the Boltz test or increase its sensitiveness through any clarification of interfering colors. The exact sensitiveness of

*From the U. S. A. Trans. Hospital.
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the test up to the present time has not been determined. It is believed to be amply sensitive to demonstrate a clear-cut easily readable reaction with pathologic positives.

Tryptophane was found to be present in 33 per cent of 525 specimens examined from general surgical and medical cases. Of 313 of these specimens which were positive for albumin 91 or 29 per cent were also positive for tryptophane. The highest incidence of tryptophane in combination with albumin of any group of specimens from similar cases was shown in the group of 25 specimens from nine different cases of degenerative diseases of the kidneys, eighteen of which or 72 per cent were also positive for tryptophane. Two hundred and twenty-two specimens positive for albumin by the usual routine tests were negative for tryptophane by Boltz test, indicating the existence of an albuminuria in which the protein molecule eliminated does not contain a tryptophane group. One hundred and sixty-nine specimens in this series were found positive for tryptophane by Boltz test, of which number 78 or 46 per cent were albumin free. This indicates the existence of a tryptophanuria as a clinical entity distinct from albuminuria.

The highest incidence of free tryptophane not in combination with albumin was found in the small group of five cases of nervous disorders such as menopause, neuritis, neurasthenia, hysteria, neuroretinitis and seven cases of surgical shock such as amputation, gunshot and other wounds, fracture dislocation, and secondary hemorrhage. The figures while meager led me none the less to the conclusion that tryptophanuria as a distinct clinical entity might most likely be found in the group of nervous and mental disorders. This conclusion has been partially verified by the examination of over 600 specimens from 101 cases of mental disease as follows: miscellaneous diagnoses 7, general paresis 7, manic depressive psychosis 7, dementia praecox 80. The average incidence of tryptophane positive specimens was slightly less than in general surgery and medicine, being but 21 per cent, this finding, however, representing almost entirely free tryptophane not in combination with albumin. Of the 101 cases examined by the test eight in the dementia praecox group have been found who consistently run a tryptophanuria with no concurrent albuminuria.

The evidence is rather strongly indicative that tryptophanuria in nervous and mental disease is most likely to occur during periods of hyperactivity. This conclusion was reached by a consideration of the findings in individual cases and is supported by the following figures. Of 350 specimens taken at random from the four halls of one ward of the United States Veterans Bureau Hospital No. 24 at Palo Alto, California, 50 specimens or slightly over 14 per cent were found positive, while of 258 specimens taken in the tub room of the same ward 79 or about 31 per cent were positive. The findings with reference to the effect of tubs and packs indicate that this treatment is more likely to increase a tryptophanuria than to diminish it.

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COMPARISON OF THE HUDDLESON SLIDE TEST WITH A MACROSCOPIC TUBE TEST IN UNDULANT FEVER*

BY HENRY WELCH PH D, AND FRIEND LEE MICKLE M S HARTFORD CONN

THE literature on the relative merits of the agglutination test for undulant fever indicates that the Huddleson test is as specific and as sensitive as the macroscopic tube test. Huddleson and Abell¹ have tested thousands of serums with the rapid (Huddleson) method and find it as accurate and specific as any other method of detecting the presence of specific antibodies for *Brucella abortus*. Damon,² Palmer and Baker³ and Lienhardt and Kitselman⁴ all report the Huddleson test as accurate as the macroscopic tube test.

These Laboratories have been reporting on the presence of agglutinins for the *Brucella* group in blood sera since November 19 1926. This work up to July, 1930, has been published^{5 6 7 8 9} and with investigational studies included some 20,500 sera examinations. The sera on reaching the Laboratories were sent to the Connecticut (Storrs) Agricultural Experiment Station where the macroscopic tube test was made first by Dr J G McAlpine and later by Dr W N Plastridge, the results reported to these Laboratories and from here subsequently to the physician. The time required caused considerable delay before the physician received his report. It was felt that by the use of the Huddleson technique, which is a considerably shorter method for diagnosing undulant fever, the test could be made in our own Laboratories at a great saving in time. Accordingly, the Huddleson rapid method has been run routinely on all specimens sent to the Laboratories for diagnosis of undulant fever for the past five months. The results obtained have been compared with the results of the macroscopic tube test made in the Storrs laboratory.

METHODS

Huddleson Rapid Method—The apparatus used in the Huddleson test has been described¹ and is easily constructed in the laboratory. For our investigation a box of 16.5 × 11 × 6 inches containing two 50-watt electric light bulbs was used. (Note Fig 1.) The top of the box was partially covered with half-inch wood five inches wide to protect the eyes of the technician reading the tests. The light bulbs were attached to either end of the box so as to be under the five-inch strip of wood. A piece of plate glass lined off in one and one-half inch squares covered the rest of the top of the box.

The Huddleson antigen is standardized for use with undiluted sera in the following amounts 0.08 cc 0.04 cc 0.02 cc 0.01 cc and 0.004 cc corresponding to dilutions of 1:25 1:50 1:100 1:200 and 1:500 in the tube test. For comparative purposes agglutinations were made out to the 1:3000 dilution. It was found possible to do this by a preliminary comparison of the

*From Bureau of Laboratories, Connecticut State Department of Health.
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Huddleson antigen in a tube test (diluted) and in a regular slide test (undiluted)

Each specimen of blood on reaching the Laboratories was centrifuged and the clear sera decanted. A portion of this sera was sent to the laboratory at Storrs for the macroscopic tube test, and the remaining portion distributed in varying amounts to produce dilutions 1:25 through 1:3000 (note Table II) on the plate glass. One drop of antigen—Huddleson from a standardized dropper was then added to each dilution. The antigen and sera were mixed with clean toothpicks and the plate glass rotated for two to three minutes. The lights were then turned on inside the box and the degree of clumping in the different dilutions estimated. Occasionally in questionable positives we found it possible to obtain more accurate readings by placing the plate glass on a black background and reading by direct light with a dissecting microscope.

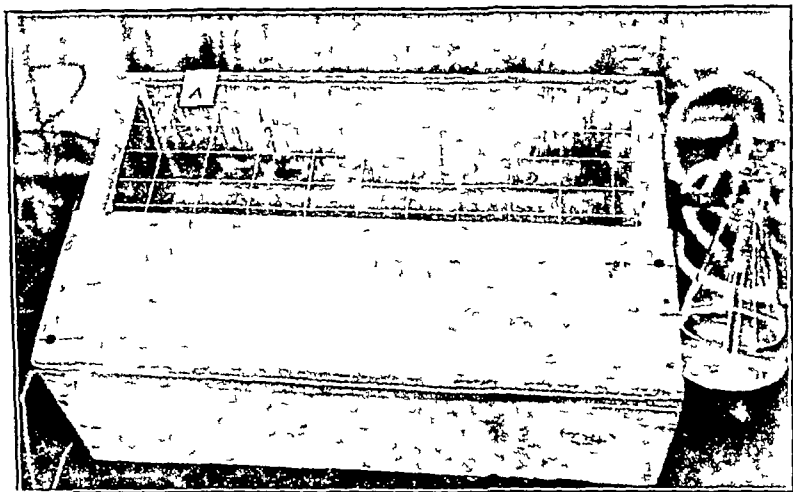


Fig 1—Apparatus used for Huddleson test

Macroscopic Tube Test—The antigen employed in the tube test was made from four strains of *Brucella abortus*, coming from different sections of the country, whose agglutinability had been carefully checked. These strains were grown for twenty-four hours on 1 per cent glycerin Fauchild's agar, and from these transplants were made upon plain Fauchild's agar and incubated forty-eight hours at 37° C. The growth was harvested with a small amount of 0.4 per cent carbolyzed saline solution and diluted for the final test to a nephelometer 1 on the McFarland¹⁰ Scale. This diluted antigen was distributed in small test tubes in 2 cc amounts and the serum added to make final dilutions of 1:25 to 1:3000 as shown in Table II. After the addition of antigen the tubes were corked and shaken, incubated for forty-eight hours at 37° C, and one reading was made on the removal from the incubator and a second after twenty-four hours at room temperature.

RESULTS

A total of 156 sera were examined. Of these sera 20 were positive from a clinical and laboratory standpoint by both tests, there were 122 negative with both tests, 10 questionable reactions with the tube test and two with the Huddleson rapid test. These results are summarized in Table I.

TABLE I

RESULTS OF HUDDLESON AND MACROSCOPIC TUBE TESTS ON 156 SERA BETWEEN AUGUST 12, 1930 AND DECEMBER 31, 1930

	WITH HUDDLESON	WITH TUBE TEST	WITH BOTH
Positive tests	20	20	20
Negative tests	130	122	122
Questionables*	2	10	10†
Repeated positives	4	4	4
Total tests	156	156	156

*Clinically negative

†Includes ten questionable tube tests two of which also were questionable with Huddleson

An examination of this table shows that there is no difference in sensitivity with either test in the positive cases of undulant fever. However, there were eight more questionable reactions with the tube test than with the Huddleson which indicates a lesser degree of specificity of this tube test. The titers in this series of eight questionable reactions in the tube test varied from 2+ agglutinations in the 1:75 dilution to 2- agglutination in the 1:25. In no cases were definite agglutinations noted in the 1:100 dilution.

In order to show the relationship of the Huddleson rapid test and the macroscopic tube test more accurately the 20 positive tests with their titers are given in Table II. In each of these cases a clinical diagnosis of undulant fever was made.

By examination of Table II it will be seen that the Huddleson test gave higher titers than the macroscopic tube test in eleven of the 20 tests. Two of the macroscopic tube tests were slightly higher than those of the Huddleson, whereas seven tests agreed in titer. It is obviously not our intention to place too much emphasis on differences in titers by these two tests. Differences in technique and technicians will probably account for it, but it is interesting that in using such a series as this that the Huddleson rapid test did seem to show titers somewhat higher than the macroscopic tube test.

DISCUSSION

According to the Illinois Undulant Fever Commission¹¹ a positive reaction in a titer of 1:100 is considered diagnostic for undulant fever. In the series of 20 positive cases reported in this paper only in one case (note sera No. 69412) was a tube test titer as low as 1:100, and in this case the Huddleson rapid test showed a much higher titer (1:1500). In our experience the titers of sera from cases of undulant fever are rarely as low as the 1:100 dilution. In all 20 cases the Huddleson test showed agglutination in the 1:1000 dilution or better. Clinical cases with low titers reported by workers elsewhere have not been encountered in Connecticut.

TABLE II

DEGREE OF AGGLUTINATION WITH HUDDLESON RAPID TEST AND MACROSCOPIC TUBE TEST ON
TWENTY CLINICAL CASES OF UNDULANT FEVER

DILUTION OF SERA		25	50	75	100	150	200	300	500	750	1000	1500	3000
57522	H	4+	4+		4+		4+		4+	4+	4+	4+	4+
	M	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
	H	4+	4+		4+		4+		4+	3+	3+	3+	+
61207	M	4+	4+	4+	4+	4+	4+	4+	4+	3+	2+	?	-
	H	4+	4+		4+		4+		4+	4+	4+	4+	4+
61923	M	3+	4+	4+	4+	4+	4+	4+	4+	4+	4+	3+	2+
	H	3+	3+		3+		3+		3+	2+	2+	1+	-
69412	M	4+	4+	3+	2+	?		-	-	-	-	-	-
	H	4+	4+		4+		4+		3+	2+	1+	1+	-
69822	M	2+	4+	4+	4+	4+		2+	?	-	-	-	-
	H	4+	4+		4+		4+		4+	4+	4+	4+	4+
70677	M	?	3+	4+	4+	4+		4+	4+	4+	4+	3+	2+
	H	4+	4+		4+		4+		4+	4+	3+	2+	2+
73790	M	4+	4+	4+	4+	4+		4+	4+	2+	?	-	-
	H	4+	4+		4+		4+		3+	2+	1+	?	-
76511	M	4+	4+	4+	4+	4+		3+	?	-	-	-	-
	H	4+	4+		4+		4+		4+	4+	4+	4+	4+
76787	M	4+	4+	4+	4+	4+		4+	4+	4+	4+	4+	4+
	H	4+	4+		4+		4+		4+	4+	4+	4+	3+
78334	M	4+	4+	4+	4+	4+		4+	4+	4+	3+	2+	-
	H	4+	4+		4+		4+		4+	4+	4+	4+	4+
79025	M	4+	4+	4+	4+	4+		4+	4+	4+	4+	4+	2+
	H	4+	4+		4+		4+		4+	4+	4+	3+	2+
79469	M	4+	4+	4+	4+	4+		4+	4+	4+	4+	4+	4+
	H	4+	4+		4+		4+		4+	4+	4+	4+	4+
79597	M	?	?	2+	4+	4+		4+	4+	3+	2+	?	-
	H	4+	4+		4+		4+		4+	4+	4+	2+	-
80158	M	4+	4+	4+	4+	4+		4+	4+	4+	4+	2+	?
	H	4+	4+		4+		4+		4+	3+	3+	2+	-
80160	M	4+	4+	4+	4+	4+		4+	4+	4+	4+	3+	2+
	H	4+	4+		4+		4+		4+	4+	4+	4+	3+
80957	M	4+	4+	4+	4+	4+		4+	4+	4+	4+	4+	2+
	H	2+	2+		4+		4+		4+	4+	4+	4+	4+
81728	M	4+	4+	4+	4+	4+		4+	4+	4+	4+	4+	4+
	H	4+	4+		4+		4+		4+	4+	4+	4+	4+
81924	M	4+	4+	4+	4+	4+		4+	4+	4+	4+	4+	4+
	H	4+	4+		4+		4+		4+	4+	3+	2+	-
82280	M	?	2+	3+	3+	3+		3+	3+	2+	2+	-	-
	H	4+	4+		4+		4+		4+	4+	4+	4+	4+
521	M	4+	4+	4+	4+	4+		4+	4+	4+	4+	4+	4+

4+ Complete Agglutination

3+ 75% Agglutination

2+ 50% Agglutination

+20% Agglutination

- % Agglutination

H = Huddleson macroscopic test

M = Macroscopic tube test

The questionable reactions noted were obtained in most instances from cases of typhoid fever or else from patients who had had typhoid vaccine within a year. Whether these reactions were cross agglutinations with typhoid or natural agglutinins for the Brucella group in the blood stream of the patient is difficult to say. The fact that the patients had typhoid fever or typhoid vaccine is at least suggestive. Gilbert and Coleman¹² report the presence of typhoid agglutinins in the blood stream of patients with febrile diseases who had never received typhoid vaccine or to their knowledge had typhoid fever. It may well be that the questionable reactions we obtained were of a similar nonspecific nature being induced by previous injections of typhoid vaccine or a previous infection with the typhoid bacillus.

Our results indicate further that the Huddleson rapid method is slightly more sensitive and specific than the tube test used for comparison. Whether these differences observed were due to the type of macroscopic tube test used or indicate a superiority of the Huddleson technique over that of the tube test cannot be stated definitely without further work with a variety of antigens and tube test techniques. However, we do feel that for our routine examinations considering the ease and rapidity of the test, the sensitivity and specificity of the antigen and the time saved in getting reports to our physicians the Huddleson rapid method is far superior to that of the macroscopic tube test.

SUMMARY

A series of 156 specimens of blood tested for the presence of agglutinins for undulant fever with a macroscopic tube test and the Huddleson rapid slide method indicates that the latter test is slightly more specific and sensitive than the former. Titers as low as 1:100 dilution in positive cases occurred in one case only with the tube test and the Huddleson in all positive cases showed typical agglutination in the 1:1000 dilution or higher. The rapidity and ease with which the Huddleson method can be carried out makes it an excellent test for laboratories where the diagnosis of undulant fever is carried out routinely.

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A PROPOSED STANDARD METHOD FOR THE EVALUATION OF FUNGICIDES*

By ADELIA MCCREA, PH D, DETROIT, MICH

TO THOSE who work with fungi, especially parasitic fungi, the problem of a safe and certain fungicide is of great importance. Much work has been done in the attempt to find or to develop effective agencies for the destruction of these pests of the field and laboratory.

In recent years, interest in this direction has rapidly increased due to widespread infection by fungi parasitic upon the human skin. These skin mycoses are encountered much more frequently at present than they were a decade ago, and doubtless the average physician has come to recognize them more readily as they become more common. This type of skin lesion bears many pseudonyms, e g, golfer's itch, student's itch, athlete's foot, and, while not fatal, it is the cause of a tremendous amount of annoyance and lowered efficiency among those so afflicted.

In an attempt to compare the reports of various workers heretofore made on the germicidal properties of substances used for combating bacteria and fungi, it became evident (a) that no standard method has been adopted by workers in this field, and (b) that practically all of the tests heretofore made *in vitro* have been based on continued contact, i e have been *fungistatic* tests rather than *fungicidal* tests. To call a substance fungicidal unless it actually kills the organism is faulty terminology.

In connection with a study of two of the most common organisms encountered in mycoses of feet and hands, i e, *Trichophyton interdigitale* Priestly and *Epidermophyton rubrum* Cast, the writer has developed a test method for fungicidal powers of agencies against these and other fungi, which it is hoped may be conveniently followed by other workers. Once such a method is established, it will be possible to compare very closely the results obtained by many workers, and should greatly decrease the amount of conflicting results in subsequent reports. In outlining the method below, an effort has been made to give all important details in full, but the writer will be glad to give any additional information which may be desired. The method follows in detail.

Medium Used—Sabouraud's original formula for glucose agar medium was used except that American ingredients were necessary† since the French glucose is not obtainable. The formula is

Glucose (Merck)	4 0%
Peptone (Witte)	1 0%
Agar-agar	1 8%

*From the Research and Biological Laboratories Parke Davis and Company.

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†For purposes of isolation and identification of organisms the French peptone Chassaigne is used but this was not practical for the large amounts used in these tests.

This is made up with tap water, sterilized fractionally in flowing steam for one-half hour on three successive days, adjusted to neutral and stored in flasks or tubed at once as desired. This medium is used throughout the tests.

Reagents—In reporting the value of any agency it is most important that the *source should be given* as well as the exact designation e.g. "Auramine" is prepared by various manufacturers and may be listed as "Auramine O," "Auramine OO," etc. the preparations often varying widely in their efficiency. Proper designation would be, e.g. "Auramine O, Coleman and Bell" or whatever the case may be.

It is also to be realized that the question of penetration is significant in any study of lethal action, and that an ineffective substance, if properly buffered may become of value, hence it is important that any and all such modifications be stated when data are given.

Physical Factors—To reduce the variation due to these factors, the following conditions are adhered to: (a) incubation in the dark at a temperature of 22° to 28° C. (b) For agar slants, test tubes 16 x 140 mm are used with a butt of $\frac{3}{8}$ to $\frac{1}{2}$ inch. (c) Spore suspensions are made on the basis of 1 culture tube of growth taken up in 8 to 10 c.c. of sterile, distilled water. This inoculum is then strained through two layers of gauze (cheesecloth) to remove mycelial fragments and prevent "clumping" of conidia. It is then tubed and used within six hours after preparation in planting the test liquids.

Fungicidal Test—This test involves exposing the heavy strained spore suspension for definite time periods to a freshly prepared solution of the desired strength of the reagent being tested. After each interval of exposure a portion of the material is transferred by loop to the surface of tubes of the standard medium usually two loops per tube. The tubes are then incubated as above described with the customary control cultures. The writer's practice is to add 0.5 c.c. of spore suspension to 2 c.c. of each test solution. At intervals of one minute, ten minutes and one hour, portions of the mixture are taken by stirring with the loop and transferring to tubes of culture medium. Other quantities or time intervals could readily be adapted to this method, but, in that event, *they should always be definitely stated* for the benefit of later workers and this holds also for the addition of one's choice of buffer or other modification of the method. Only by such cooperation may we eventually overcome the lack of harmony in the results of workers interested in this matter.

To give a concrete illustration, suppose e.g. that a test is being made of the effect of gentian violet upon say, a *Trichophyton* culture. The procedure would be as follows. 0.10 gm. of the dye is taken up in a few drops of alcohol and diluted with water to 25 c.c. giving a solution of 1 to 250. From this stock solution one readily prepares a dilution series of 1-250 1-500 1-1000 and 1-5,000 which is sufficient range for a preliminary test. Each dilution is arranged in its respective tube 2 c.c. per tube. To it is added 0.5 c.c. of spore suspension prepared as described above. Transplants are made to two tubes of the standard medium after one minute, ten minutes and one hour and placed in the dark cupboard. By "staggering" the intervals the whole series of four dilutions and three time intervals can be completed in less than one

and one-fourth hours. Within the next forty-eight hours, often in twenty-four hours, growth will indicate which dilutions are ineffective, e. g., growth may be normal at 1:5,000 for all three time exposures but 1:1,000 show growth only after one minute and ten minutes. This gives a middle ground from which to plan the final test. From the stock solution, kept in the refrigerator, dilutions are prepared of 1:600, 1:800 and 1:1,000 which are then tested exactly as before and give a final rating, if good judgment has been used in the dilution series chosen. Table I serves to illustrate the method.

FUNGICIDAL TEST

Organism *Trichophyton A*
Test substance Gentian violet (C & B)

	DILUTION	ONE MINUTE	TEN MINUTES	ONE HOUR
Preliminary Series	1:250	- -	- -	- -
	1:500	- -	- -	- -
	1:1,000	+ +	+ +	- -
	1:3,000	+ +	+ +	+ +
Final Series	1:600	- -	- -	- -
	1:800	+ +	- -	- -
	1:1,000	+ +	+ +	- -

Interpretation: Gentian violet is fungicidal for this organism in one minute at 1:600, in ten minutes at 1:800, and in one hour at 1:1,000, but permits normal growth after an exposure of one hour at 1:3,000.

It is recognized (a) that the addition of the spore suspension to the test fluid increases the dilution, and (b) that in borderline cases additional time intervals may occasionally be desirable. However, the foregoing method gives a very good idea of the fungicidal activity of test substances and, from a pragmatic point of view, has proved most helpful in establishing comparative values.

A report is in progress of results obtained under the above method with a group of the aniline dyes and with various other materials, which it is expected will be published in the near future. This will show a compilation of the data obtained by this method in tests of approximately forty substances. Eleven organisms were used during these studies. When checked by application to the actual "ringworm" lesions in the guinea pig, the above "Fungicidal Test" was found to be of greater practical use than any other means of evaluation tried.

BY BURGESS GORDON, M D , PHILADELPHIA, PA

A detailed diagram of a mechanical apparatus. It features a central rotating disk with a pointer labeled 'E' and a scale around its circumference. To the right of the disk is a circular dial with a needle, labeled 'H' and 'M'. The entire assembly is mounted on a base labeled 'D'. A large curved pipe or hose is connected to the top of the disk, labeled 'C' and 'A'. Another curved pipe is connected to the bottom of the dial, labeled 'B' and 'F'. A small rectangular component is labeled 'L'. A vertical rod or shaft is labeled 'K'. The dial has a needle pointing towards the 'M' mark.

operation and makes calculations difficult. The question of faulty procedure may be raised even with careful technique and efficient apparatus because a graphic record is not available. In addition the usual devices for controlling and estimating the amount of air passing into the chest are not entirely satisfactory.

*From the Medical Service of Dr. Thomas McCrae and the Department for Diseases of the Chest, Jefferson Hospital, Philadelphia, Pa.

pressure gauge *H* (recording up to 1050 cc) shows the amount of air in the reservoir. The variations in negative and positive pressure are recorded on a paper chart (Fig 2) by means of a set-up consisting of pet cocks, bellows, connecting rod, gear box, tracing needle and kymograph. The chart holder *G* and the pressure gauge *H* are tilted at convenient angles so the operator may observe the variations without difficulty.

The instrument is operated as follows. The chart is placed in position on the kymograph, pet cock *A* is closed, pet cock *B* opened. The air reservoir is filled by operating the compression pump *L*, pet cock *B* is then closed. After preparing the site of operation in the usual manner pet cock *C* is opened, ink applied to the tracer *E* and the kymograph started by elevating the release lever *K*. The pneumothorax needle *F* is forced carefully into the pleural cavity and as entrance occurs the tracer *E* will swing to the left (to the right if the

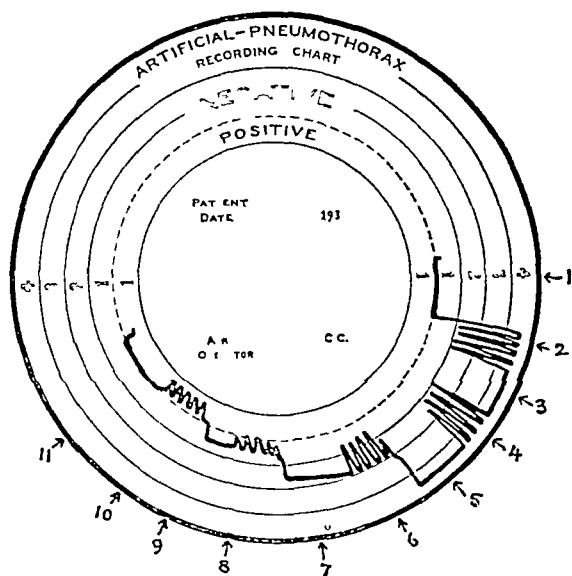


Fig 2—The irregular line represents different stages in treatment. During periods 1 3 5 7 9 pet cock *C* was closed. Periods 2 4 6 8 show negative respiratory movements recorded while pet cock 4 was closed and pet cock *C* open. Period 6 indicates an approach to positive pressure and period 9 shows that positive pressure has occurred. The operation was terminated during period 11.

pressure is positive) and the degree of pressure will be recorded on the chart *G*. Pet cock *C* is closed and the lever on pet cock *A* is opened to the first numeral on the dial. As this occurs air will pass into the pleural cavity, the rate and amount being shown on the gauge *H*. The rate is increased by moving the lever on pet cock *A* to numerals 2, 3 or 4. At intervals pet cock *A* is closed and *C* is opened in order to determine the approach to positive thoracic pressure. When the tracer *E* passes to the right of the dotted line on the chart, the indication is that positive pressure has occurred. At this point treatment should be discontinued by closing the release lever *K*, pet cocks *A* and *C* and withdrawing the pneumothorax needle *F*. The total amount of air that has entered the chest is determined by subtracting the number indicated by the hand on gauge *H* from the figure noted at the beginning of the operation. This is

written on the chart, with the date, patient's name and that of the operator. About once every three weeks for antiseptic purposes, 2 cc of alcohol should be dropped into the air filter U.

The chief features of the instrument are the exclusion of manometers, glass jars, and stoppers and the use of a device for recording variations in the degree of thoracic pressure. These simplify operation and reduce the source of error.

The instrument is manufactured by the George P. Pilling and Son Company, Philadelphia, Pa.

A SIMPLE CLINICAL PROCEDURE FOR THE DETERMINATION OF UREA IN URINE BY MEANS OF HYDROLYSIS*

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IN 1929 we¹ described a method for the determination of urea in blood by digesting the protein-free blood filtrate under pressure in a closed tube. We have now extended the use of this method to the quantitative determination of urea in urine. The details of the procedure are here described.

Principle—The ammonia is removed from the urine with permuted according to the procedure of Folin and Bell.² The ammonia-free urine is digested under pressure in an acid medium for ten minutes. The resulting ammonium salt is nesslerized directly in the digestion tube and compared in the colorimeter to a known standard.

Procedure—Three cc of urine are placed in a 200 cc Erlenmeyer flask to which are added 3 to 4 grams of permuted and 27 cc of water. The mixture is shaken for five minutes and filtered through filter paper. Five cc of the filtrate are diluted with water to 100 cc in a volumetric flask. Five cc of this diluted filtrate (1:200 dilution) are placed in a urea pressure tube to which is added 1 cc of approximately normal H_2SO_4 . The stopper is rinsed with 1 cc of water and tightened in the neck of the tube. The tube is then placed in an oil bath which is heated at 145°-150° C for ten minutes. The tube is removed from the oil bath and cooled. It may be cooled immediately under running tap water without any danger of breaking the tube. About 15 cc of water are then added followed by 3 cc of modified Nessler solution.³ Water is added to the 25 cc mark and the contents well mixed. It is then compared in the colorimeter against a standard containing a known amount of ammonia.

The standard is the same as used for the determination of urea in blood. It contains 0.3 mm of nitrogen in 5 cc of solution. It is prepared by placing

*From the Biochemical Laboratory of I. Baron Hospital.

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†The pressure tubes and a special oil bath may be obtained from Filmer and Amend, New York.

5 c c of the standard ammonium sulphate in a 100 c c volumetric flask containing about two-thirds its volume of water. To this are added 12 c c of Nessler solution and water to the 100 c c mark.

The standard is placed in the colorimeter at 20 mm. If the color of the unknown is much deeper than that of the standard due to a greater concentration of ammonia, a portion of the unknown solution is transferred to a narrow tall graduated cylinder and diluted with dilute (1:10) Nessler solution until the color of the unknown approximately matches that of the standard.

Calculation of Results

$$\frac{6000}{R} \times \text{Dilution} = \text{Mg nitrogen in 100 c c of urine}$$

R = Reading of unknown

Dilution = Dilution of Nesslerized solution

This method of hydrolysis of urea also breaks down other substances than urea and therefore gives slightly high results. We therefore do not advocate the use of this method for determination of urea in urine when absolute values are desired, such as, for example, when studying the nitrogen partition. However, when a nitrogen partition is to be made, in spite of the somewhat higher values, we prefer this method to the methods using urease hydrolysis and we find it satisfactory for clinical purposes.

The pressure tubes, after use, are washed with dilute nitric acid and thoroughly rinsed with water. Omission to do this, will leave some Nessler reagent in the tube which will produce cloudy solutions. The tubes after being thoroughly washed are placed in a rack with their mouths down in such a manner that the stoppers are held loosely halfway in the tubes thus allowing the water to drain.

Nessler solution should never be added to a warm solution and preferably is to be added rapidly. When these precautions are observed a clear solution will always be obtained.

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AN IMPROVED MICRO KJELDAHL METHOD*

By J W CAYETT, PH D, MINNEAPOLIS, MINN

OWING to the necessity for a Kjeldahl method that could be used to analyze small quantities of nitrogen which were present in relatively large volumes of liquid and still maintain an accuracy similar to that of the macro Kjeldahl, the following method was devised This method is capable of

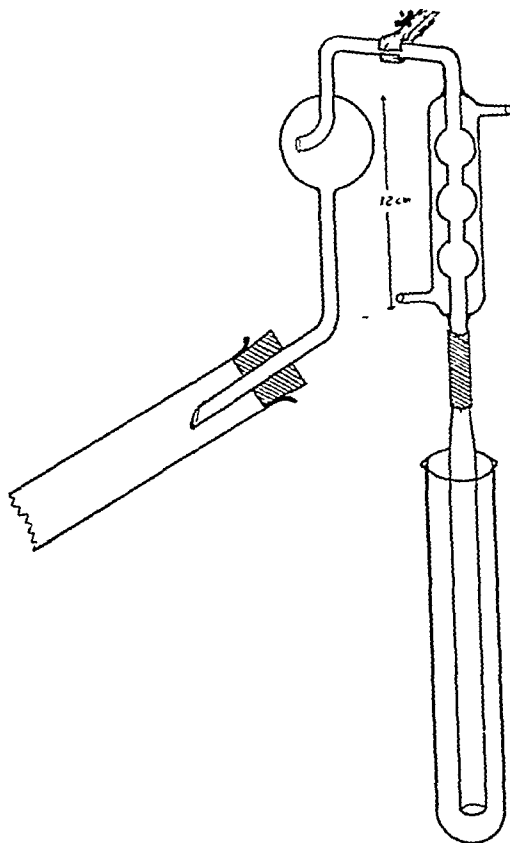


Fig 1

determining 0.5 mg to 14 mg of nitrogen with an accuracy of 0.006 mg or less. Bumping and sucking back have been eliminated due to modifications of the apparatus. Also the chance for a leak during distillation has been greatly reduced and the whole apparatus may be easily cleaned as it is all glass. The distillation period has been shortened to ten minutes and the titration is carried out in a manner which increases its accuracy.

*From the Laboratory of Physiological Chemistry, University of Minnesota.
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METHOD

The sample to be analyzed is placed in a 300 c c Kjeldahl flask and 4 c c of concentrated sulphuric acid and a few drops of 5 per cent copper sulphate solution added (A half gram of sodium sulphate or a few drops of H_2O_2 increases the speed of digestion) The digestion is carried out in a hood After digestion is complete and the flask has cooled, 40 c c of distilled water is added Four glass beads and about 10 particles of 20 mesh zinc are added to prevent bumping The mixture is then underlaid with 12 c c of 1 to 1 carbonate-free sodium hydroxide The flask is connected by a new *soft* rubber stopper to the special Kjeldahl bulb which is suspended from a ring-stand by a burette clamp The special Kjeldahl bulb including a small condenser is shown in Fig 1 A $1\frac{1}{2}$ cm glass tube constructed at the upper end and 25 cm long is attached to the lower part of the condenser by a piece of rubber tubing This glass tube extends into a 22×3 cm test tube which contains 10 c c of standard acid and a drop of methyl red solution The test tube is held by a clamp so that the delivery tube extends to the bottom at first but after the distillation is in progress it is kept about 1-2 cm below the surface The standard acid used is N/10 or N/50 depending on the amount of nitrogen in the sample being analyzed After all connections are complete, the flask is rotated thus, mixing the solution with the NaOH Twenty-five c c of water is then distilled over

The titration is always carried out with N/50 carbonate free sodium hydroxide With the smaller quantities of nitrogen, 0.5 to 2.8 mg, when N/50 standard acid is used the titration is made with a 10 c c burette calibrated to 0.05 c c, otherwise an ordinary accurately calibrated 50 c c burette is used The acid in the test tube is stirred during the titration by bubbling air which has passed through weak acid and alkali through it By this method of stirring the solution during the titration, one can easily determine an end-point to within 0.02 c c of N/50 NaOH when using the 10 c c burette Less time is required than if the solution is stirred with a stirring rod or shaking and the end-point is easier to read

SUMMARY

The Kjeldahl bulb is fused on to the condenser giving an apparatus which is compact, easy to clean and eliminating possibility of leaks in connections The use of smaller quantities of reagents and the presence of glass beads and 20 mesh zinc eliminates bumping The sucking back is avoided by use of a large delivery tube and with moderate care a determination is never lost The method has the advantage that a number of determinations can be run at once having an accuracy similar to the macro Kjeldahl on a sample almost as small as is ordinarily used for the micro Kjeldahl

NOTE The special Kjeldahl apparatus may be obtained from Arthur H Thomas Co, Philadelphia

A NEW AND SIMPLIFIED TECHNIC OF SURGICAL PHOTOGRAPHY*

BY L. F. PIEPCE PH D, CARMEL CALIF

FOR a considerable period photography of operative surgery has been a topic of interest. Frequent articles appear setting forth various items of technic and equipment. The writer pleads no interest beyond that of the photographic amateur who is interested in any discussion tending to raise the level of teaching scientific subjects.

Observation of two reels of surgical movies brought a curious thought to mind with the result that the reels were run again with a stop watch. The two reels of fifteen minute length checked within forty seconds of each other in that the field of operations was masked for a total of six minutes by the hands of either the surgeon or his assistant, a total loss of 40 per cent in the picture. When we recall that good moving picture equipment complete for such work cannot be had for less than approximately three to six hundred dollars with an actual outlay of about eight to twelve dollars for film used to make a picture of six to ten minutes' duration, the cost item mounts rapidly enough that a 40 per cent loss is appreciable.

It is the understanding of the writer that actual surgical technic is very definitely taught to the medical student and later to the graduate. There is of course justification for moving picture records of a great master doing each classical operation. From a teaching standpoint, there is even justification for multiplication of these pictures to show the various modifications of these technical items. But for the daily photographic need of the surgeon, it would appear that his real need is to have a permanent photographic record of the unusual or anomalous details which come to light at the operating table. If these details can be readily recorded without the aid of a professional photographer and preferably by a nurse in a simple manner with a minimum of special equipment and at a nominal cost it would seem that a desirable end had been reached.

For this work film packs or plates must generally be avoided in favor of roll film. The lens must be capable of working at large aperture to shorten exposure. The focal length must be short to obtain maximum focal depth and avoid extremely critical focusing. The shutter should be of the iris type rather than focal plane in order to use the camera for making records of gross specimens with extremely small aperture and long automatic exposure and thus obtain fine detail at different levels. Film stock should be obtainable at low cost from any good camera supply store and the camera should be simply and reliably made and capable of being loaded as often as need be by a person having but a slight knowledge of a camera. Finally, the finished negative should be capable of enlargement without loss of detail to five by

*From the Grace Deere Vella Metabolic Clinic
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seven inches for record and to lantern slide size for projection. If in addition to this, the camera can be used for amusement, all the better for the individual who gets pleasure from the pictorial hobby.

Until a few months ago, there has been but one camera in the market which even began to meet these requirements. Because however, of its usual focal plane shutter equipment, it is not desirable for slow exposures at small aperture of gross specimens. Further, the cost has been considerable, the film is not readily obtainable all over the country and loading the camera is, to put it mildly, formidable.

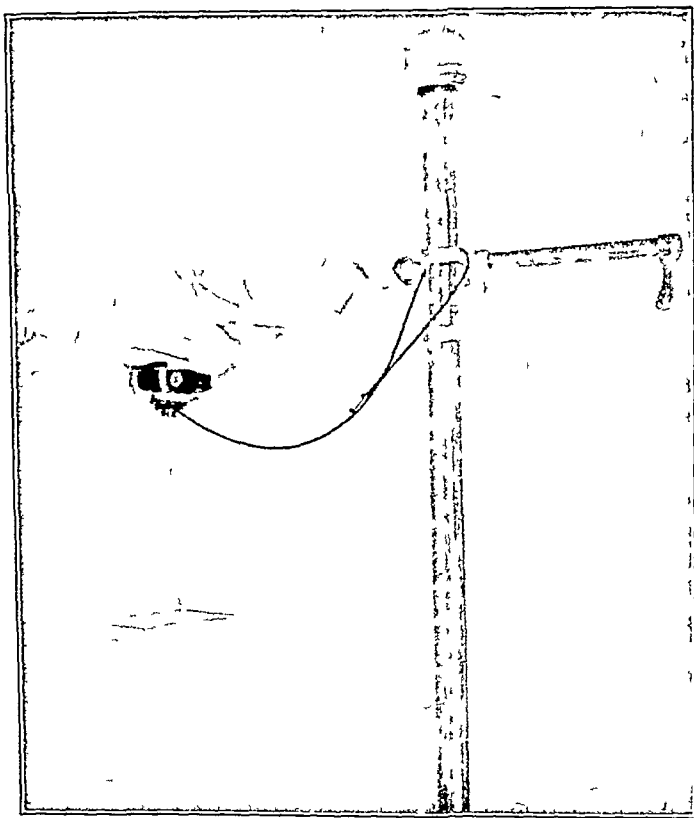


Fig 1—Camera and pyramid iconometer as supported on lamp standard

The optical firm of Carl Zeiss, Inc., has but recently produced a camera which really meets these needs in every way and at such a cost that total equipment for taking a rapid succession of stills of this kind may be assembled for well under a hundred dollars which will "cover" an average operation with a film cost of fifty-five cents and a slide cost of around four dollars if wanted.

Accurate location of the field of view is a problem, in that it is undesirable to have the camera operator leaning over the patient to look squarely down. Mirrors would add to complexity and confusion. The problem is met satisfactorily by a readaptation of the iconometer commonly used photo-

graphically on the continent. A felt padded metal collar with a constriction fitting is clamped on the shutter barrel of the "Kohlin" camera. From this four wires extend downward at angles to join a rectangular wire frame seven and one-half by ten inches which is conveniently located at a distance of thirteen inches from the lens. The lens being the tessar f 35 and fitted with the 1×24 proxar auxiliary and the shutter at maximum extension the plane of focus is at twenty inches. The frame must therefore approach to a distance of seven inches from the desired object in the wound. An ordinary six inch hemostat held vertically by the operator makes estimation of the last inch a simple matter and the frame is at no time in danger of touching anything. The frame is preferably wiped with alcohol before use. The entire outfit is suspended by means of a rubber covered four finger condenser clamp pointing down from the horizontal arm of almost any convenient therapeutic lamp standard readily found in the hospital. The actual lamp is of course dis-

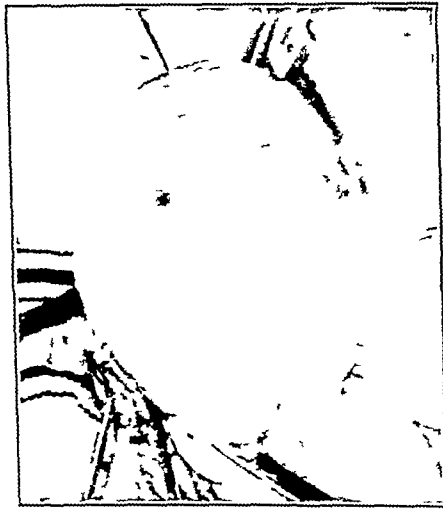


Fig 2—Supravaginal amputation of fibroid uterus. Tumor delivered and clamps applied to broad ligaments.

mounted. The entire set-up is shown in Fig 1. Thus at the call of the surgeon who steps to one side by six to twelve inches, the stand is wheeled into place, the camera lowered to the proper distance, maneuvered to center, the shutter tripped by means of a long cable release and the entire outfit pulled clear. The time for all this is not in excess of a second and a half.

If the surgeon uses a bit of sterile vaseline and free powdering of the hands with sterile zinc stearate visibility of the hands is much enhanced.

In view of the many colors encountered which are not registered on ordinary orthochromatic film panchromatic emulsion is called for and may be readily obtained at any photo supply counter through G. Gennert, Inc. of New York, the agents of the Imperial Dry Plate Works of England. At present no firm in this country is making film of this kind in the ordinary roll stock. Use of this emulsion gives excellent color separation without the use of filters. The speed of the emulsion is 'par'. Processing is simple if we emphasize to our finisher that the film must be handled either in entire dark-

ness or under the Wratten safelight. In our work we found that the fine grain borax developer formula of Eastman gave the best results by far and we used it in full strength for ten minutes at 65° F. A retail cost of fifty-five cents per roll of sixteen exposures is not excessive.

Exposures for this work must be measured with great care. The exposure meter of D. E. Meyer is very good and gives satisfactory results. We found however that we were able to expose preferentially to emphasize a given part of the wound by the use of the electric photometer of Bell and Howell. The instrument is simple and most exact. Use of such a device makes the difference between exact measurement of actinic light and pure guesswork.

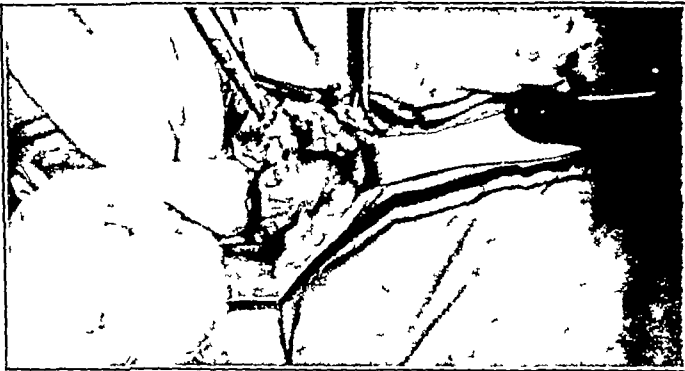


Fig. 3—Stump in abdominal wound after removal of uterus containing fibroid.

In our work given herewith in plates 2 and 3 exposures of $\frac{1}{50}$ second at f 3.5 were used with the regular nonshadow lamp found above the table. Slight variations were made for preferential emphasis. Repeated trials were made with different operators and in every case the concern of the surgeon gave way to pleased amazement and perfect cooperation at the end of the third picture. The entire time required for fourteen pictures during an hysterectomy was seventeen seconds which in no way can be said to interfere with the course of the operation.

In conclusion, we wish to repeat that our entire object has been to present a photographic technic for surgery which is simple, exact, comprehensive and finally most economical.

Drs. H. C. Ostrum, M. M. Doris, and R. J. Pickard are thanked for the kindly aid and cooperation which made this work possible.

A NEW PETRI DISH HOLDER FOR COUNTING AND FISHING COLONIES*

BY NICHOLAS KOPELOFF, PH D, AND NATHAN BLACKMAN, B S,
NEW YORK, N Y

TRADITION dictates the counting and fishing of bacterial colonies as carried out in various research, diagnostic, dairy, and public health laboratories. Little progress has been made in counting bacteria beyond the introduction of the glass counting plates devised by Jeffer, Wolfhugel and others. The accepted procedure is to superimpose the etched glass counting plate on the bottom half of the Petri dish. Then, with the aid of a hand magnifier, the colonies are counted against a dark background such as a black table top for example, or the Petri dish is held at eye level against a source of light, daylight or artificial. When the Petri dish is held at eye level the colonies in the depths of the medium are more visible and the counting process becomes more accurate. However, the hands tire rather easily in this awkward position and one cannot employ a mechanical counter because both hands are already occupied. While there is an expensive apparatus on the market that permits free use of the hands its magnifying lens fails to bring out small colonies with sufficient clarity.

Similarly, in fishing colonies the attempt is usually made to work under aseptic conditions which frequently necessitates artificial illumination from above. In any event it is difficult to see clearly into the depths of the medium and often there is great hardship in fishing from the edges of given colonies as is necessary in microbial dissociation studies.

A simple Petri dish holder devised by one of us (Blackman) is herewith described. It may be attached to the upright stem of an ordinary iron support. The holder is a metal frame which holds the Petri dish in place at any height and at any angle, thus permitting free use of the hands. Plate 1 is a photograph of the front view and Plate 2 is a rear view.

The specifications of the Petri dish holder are as follows. A square piece of galvanized iron sheeting 5 inches wide and $\frac{1}{32}$ of an inch thick has an edge folded at right angles to the depth of $\frac{3}{4}$ of an inch. It is painted black. A round hole exactly the size of a Petri dish is cut in the metal sheet. A Jeffer's glass counting plate is then fastened on the inside of the frame. Four $\frac{3}{8}$ inch stay springs (A) at the rim of the hole hold the Petri dish in place. The edges of the stay springs are bent up to hold the bottom half of the Petri dish in place. Two tension springs (B) $1\frac{1}{4}$ of an inch on a $\frac{3}{4}$ inch post clamp the Petri dish in place. The metal frame is moved up and down on the upright

*From the Department of Bacteriology, Psychiatric Institute and Hospital, New York City.
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stem of an iron support by means of a slide joint (*C*). A tight fitting swivel joint (*D*) allows the frame to be tipped to any desired angle.

The construction of this Petri dish holder is simple and the cost of materials almost negligible. The Petri dishes are firmly fixed.

The advantages of this Petri dish holder may be thus briefly summarized.

1 The hands are left free for use in handling a mechanical counter, fishing, etc.

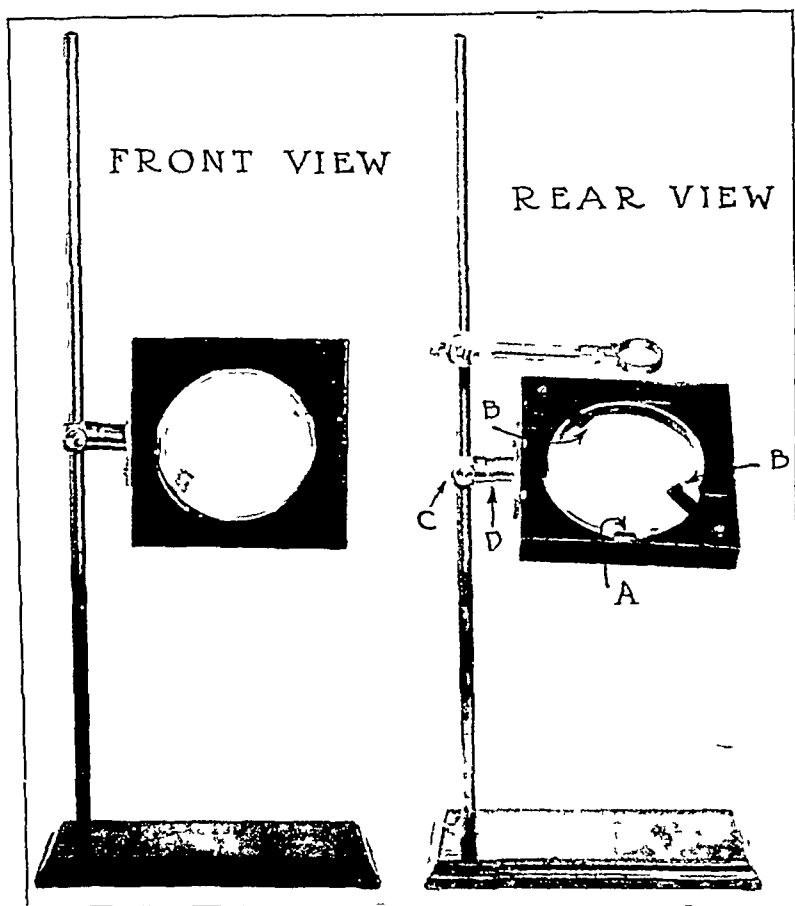


Fig 1

Fig 2

2 Petri dishes are clamped in such a way that either daylight or artificial illumination is allowed to penetrate the depths of the medium.

3 Counting or fishing can be done at any desired angle or eye level. When fishing the frame is tilted at an angle of 45° and a microscope lamp with blue glass filter is used.

4 There is no movement of the Petri dish to make for inaccuracy.

In order to facilitate direct examination of the colonies with a magnifier one of us (Blackman) has devised an adjustable holder for the magnifying

*Thanks are due Mr. E. L. Phelan for his skillful execution of the design.

lens This also is attached to the upright of an iron support Plate 3 is a front view photograph

The magnifying lens is held in a brass holder consisting of the following parts The binding screw (A) permits the holder to be fixed at any level A stud (B) has a tension screw which holds another stud (C) in place at right angles to it Stud C is moved back and forth on thread of screw (D) which

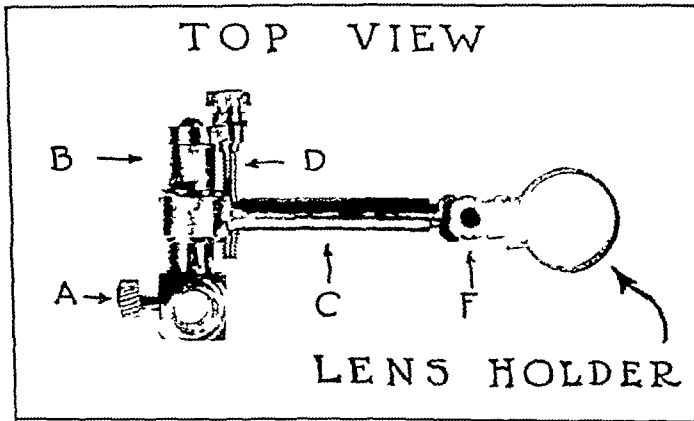


Fig 3

facilitates fine adjustment A friction joint within stud C supports the lens holder (E) which has a knuckle joint (F) permitting it to move in a semicircle

SUMMARY

An adjustable Petri dish holder and magnifying lens holder are described which are of economical construction They leave the hands free for the use of a mechanical counter or for fishing colonies Daylight or artificial light may be employed

A SIMPLE PRESUMPTIVE TEST FOR AGGLUTINATION WITH ORGANISMS OF THE ABORTUS-MELITENSIS GROUP*†

By RUTH GILBERT, M D AND MARION B COLFMAN, B S, ALBANY, N Y

THE constantly increasing recognition and, possibly, incidence of undulant fever necessitate a careful evaluation of the agglutination reaction upon which the diagnosis is at present so dependent. The specificity of this test with organisms of the abortus-melitensis group is obviously determined by results obtained in the examination of sera from (1) cases of undulant fever, (2) cases of other disease, particularly those accompanied by a febrile reaction, and (3) normal individuals. The incidence of such reactions has already been reported by many observers,¹⁻¹⁷ but only a few have had the opportunity to examine large numbers of specimens. The potential value of testing with B abortus all sera submitted for the complement-fixation test for syphilis prompted the development of a practical and economical technique for this purpose. The macroscopic slide method of Huddleson¹⁸ was first considered, but while this gives results very quickly, the testing of five dilutions of each serum requires the same number of pipettes and almost as much time as the routine macroscopic-tube method. The following procedure has, in our experience, proved most economical of both time and glassware.

Technic of the Presumptive Test—At the time sera for the complement fixation or other tests are measured, 0.01 cc is pipetted into a properly labeled 11 by 75 mm tube. These tubes are arranged in serial order in racks and empty tubes placed behind them. With a 10 cc pipette graduated in tenths, 0.6 cc of a suspension of B abortus (killed by heating at from 60 to 65° C and preserved by addition of 0.1 per cent formalin) of one half the density of barium sulphate standard¹⁹ No. 3 are measured into both the empty tubes and those containing serum, thus providing a serum dilution of 1:60 in the latter. The racks are shaken vigorously to mix the serum and suspension of organisms. It was found by calculation that a 19 gauge needle, from which the tip has been removed by filing, when attached to the barrel of a 1 cc syringe fitted with a rubber bulb, delivers approximately 0.019 cc per drop. Therefore, by the addition in this way of two drops of the 1:60 serum dilution from the tube in the front row to 0.6 cc of suspension in the tube behind it, a dilution of approximately 1:900 is obtained. Thus, a low dilution is provided for weakly reacting sera, as well as a dilution sufficiently high to be above the range of prezone reactions. After the two drops are delivered, the excess of the 1:60 dilution is replaced in the tube from which it was taken.† Before the next specimen is diluted, the syringe and needle are rinsed first with tap water and then with 0.85 per cent salt solution, both of which are contained in large jars so that only a negligible amount of serum is carried from one series of dilutions to the next. After being shaken, the tests are placed in a 55° C in

*From the Division of Laboratories and Research, New York State Department of Health, Albany.

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†Preliminary results presented at the meeting of the American Society of Clinical Pathologists, Detroit, Michigan, June 21, 1930.

‡When the presumptive procedure was first adopted dilutions of 1:20 and 1:320 were tested. The change was made because reactions in dilutions lower than 1:80 are of questionable significance and the prezone has been found in at least one instance to extend through the 1:320 dilution.

cubator for four hours, and left in the refrigerator overnight before being read. When ever a reaction is observed in either the 1:60 or 1:900 dilution or both, the serum is retested in dilutions ranging from 1:10 through 1:5000.

This simple procedure made it possible to examine 81,848 sera received for the complement-fixation test for syphilis. Three hundred and sixty-nine or 0.4 per cent of these, when retested with the usual amounts of serum, were found to react in a 1:80 or higher dilution. While most of the histories refer to the symptomatology of syphilis, presumably only a small proportion of such specimens are from cases of febrile disease and many of the reactions are no doubt due to previous infections with organisms of the *abortus-melitensis* group. In 44 instances or 0.05 per cent of those in which specimens were tested a diagnosis of undulant fever was indicated. These findings are in sharp contrast to the results of the examination of 1,186 sera submitted for the agglutination test with *B. typhosus*. Of this number, 70 or 5.9 per cent gave reactions in a 1:80 or higher dilution and 57 or 4.8 per cent were from patients found to have symptoms of undulant fever. Furthermore, in the examination of 1,255 specimens accompanied by a request for the test with *B. abortus*, 145 or 11.5 per cent gave reactions and 102 or 8.1 per cent were from probable cases of undulant fever. A summary of the reactions obtained with *B. abortus* in a 1:80 or higher dilution, correlating the degree of agglutination and the clinical manifestations, is given in Tables I, II, and III.

TABLE I

CLASSIFICATION, ACCORDING TO CLINICAL MANIFESTATIONS, OF 369 SERA WHICH AGGLOUTINATED *B. ABORTUS* IN A 1:80 OR HIGHER DILUTION, AMONG 81,848 SPECIMENS SUBMITTED FOR THE COMPLEMENT FIXATION TEST FOR SYPHILIS

	TOTAL	DEFINITE REACTION IN DILUTIONS OF							
		1:10,000	1:5000	1:2500	1:1200	1:640	1:320	1:160	1:80
Sera giving reactions	369	1	3	5	11	32	49	76	192
Undulant fever definitely diagnosed	14	1	1	1	2	2	4	2	1
Symptoms of undulant fever, definite diagnosis not made	30			1	3	6	4	6	10
No history of undulant fever reported by physician	69			1	1	5	12	15	35
No information or insufficient for drawing conclusions	256		2	2	5	19	29	53	146

The infrequency of agglutination reactions with *B. abortus* in 1:80 or higher dilutions of sera from individuals with no history of undulant fever confirms previous reports on the diagnostic significance of this test. The relatively large number of instances in which a diagnosis of undulant fever has been made as a result of testing for agglutination with *B. abortus* sera submitted to be examined for evidence of typhoid fever or syphilis i.e. 101 instances in which the possibility of undulant fever had not been considered by the attending physician before the test was made not only justifies the procedure but indicates that the syndrome of infection with organisms of the *abortus-melitensis* group is still frequently unrecognized.

TABLE II

CLASSIFICATION, ACCORDING TO CLINICAL MANIFESTATIONS, OF 70 SERA WHICH AGGLUTINATED
B ABORTUS IN A 1 80 OR HIGHER DILUTION, AMONG 1,186 SPECIMENS SUBMITTED
FOR THE AGGLUTINATION TEST WITH B TYPHOSUS

	TOTAL	DEFINITE REACTION IN DILUTIONS OF							
		1 10,000	1 5000	1 2500	1 1200	1 640	1 320	1 160	1 80
Sera giving reactions	70	2	5	7	17	18	12	8	3
Undulant fever definitely diagnosed	44	2	5	4	11	10	7	4	1
Symptoms of undulant fever, definite diagnosis not made	13			1	3	3	4	1	1
No history of undulant fever reported by physician	1					1			
No information or insufficient for drawing conclusions	12			2	1	4	1	3	1

TABLE III

CLASSIFICATION, ACCORDING TO CLINICAL MANIFESTATIONS, OF 145 SERA WHICH AGGLUTINATED
B ABORTUS IN A 1 80 OR HIGHER DILUTION, AMONG 1,255 SPECIMENS
SUBMITTED FOR THIS TEST

	TOTAL	DEFINITE REACTION IN DILUTIONS OF							
		1 10 000	1 5000	1 2500	1 100	1 610	1 320	1 160	1 80
Sera giving reactions	145	4	5	12	21	46	22	19	16
Undulant fever definitely diagnosed	81	4	3	5	17	27	16	7	2
Symptoms of undulant fever, definite diagnosis not made	21			5	2	8	2	1	3
No history of undulant fever reported by physician	3				1			1	1
No information or insufficient for drawing conclusions	40		2	2	2	10	4	10	10

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M D , ABSTRACT EDITOR

PNEUMOCOCCI, Rapid Method of Typing, Brown, M H Am J Pub Health 21 669, 1931

1 A representative sample of sputum should be obtained, if necessary, waiting until the patient expectorates material directly from the bronchial tract instead of saliva or pharyngeal secretions. A surprisingly small amount of actual sputum is necessary. The sample should be obtained in a clean sterile container, free from any preservative.

2 Wash the sputum at least three times by swirling the mass of sputum around in sterile saline contained in three separate Petri dishes. This tends to remove extraneous microorganisms. Following this, the sputum is thoroughly emulsified in saline, and in order to facilitate this, a 5 cc syringe and a needle of large caliber are very useful. At least 1 cc of emulsified sputum is then injected intraperitoneally into a mouse, using a needle of small caliber. As a matter of routine, two mice are injected.

3 In three hours a peritoneal puncture is performed, using a capillary pipette made by drawing out a piece of small glass tubing in a flame. The finer the pipette, the more satisfactory it is for this purpose. Grasp the mouse by the loose skin of the neck between the thumb and index finger, with the back of the mouse across the palmar surface of the hand, and made secure by looping the tail between the ring and little fingers. The abdomen of the mouse being thus well exposed, the capillary pipette is passed into the peritoneal cavity and by capillary traction fluid will be seen extending into the pipette. The pipette is then removed and the mouse is kept for further observation.

4 The surface of a well cleaned glass slide is divided into four sections, using a glass pencil. On each of these four sections is placed a small drop of the peritoneal exudate from the pipette. With the first of the four drops of peritoneal exudate is mixed a drop of saline which is then spread thinly and allowed to dry. To the second drop of the exudate, a drop of Type I agglutinating serum diluted 1 in 10 is added and the mixture is spread thinly and allowed to dry. Similarly, the third drop of exudate is mixed with a drop of Type II agglutinating serum, diluted 1 in 10, and is spread thinly and allowed to dry, and to the last drop of exudate a drop of Type III agglutinating serum diluted 1 in 5, is added and the mixture is spread thinly and allowed to dry. The films are then fixed by passing the slide two or three times over a moderate flame. (For convenience one usually labels each of the above four sections as Sal, I, II, III with red pencil.)

5 The slide is then stained for one minute with basic fuchsin stain. The stain, which should be fresh, is made by adding 10 cc of a saturated alcoholic solution of basic fuchsin to 90 cc of distilled water, and filtered through paper. (The saturated alcoholic solution is made by adding 10 gm of basic fuchsin to 100 cc of absolute alcohol.) Other stains such as methyl violet may be used, but basic fuchsin has been found to be the most satisfactory. Wash off the stain gently with water, blot dry and examine under an oil immersion lens. The saline suspension is examined first and serves as a control. The character of the microorganisms is then determined. Frequently a pure culture of a lance shaped diplococcus is found. The other sections are examined in sequence, and definite clumping of lance shaped diplococci in one of the sections serves to denote the type. There may be many clumps consisting of only a few organisms or a few large clumps covering several microscopic fields. In examining for clumps one should not be misled by microorganisms, such as gram negative cocci, which tend to occur in clumps, or the grape like clumps of staphylococci. These may be distinguished by being round or coccoid in shape in contrast to the lance shaped diplococcus of pneumonia. The so called capsule may be quite marked, especially in purulent exudates such as obtained from the peritoneum of the mouse. Type III pneumococci usually show very

marked capsules, often so much so that the organisms themselves are seen with difficulty and the clumps are not usually packed so tightly together as are Type I or Type II organisms. Further, in a clump of Type III pneumococci one may see a very fine thread like matrix extending from organisms to organism.

Usually the sputum is well digested in the peritoneal cavity of the mouse within three hours, especially in Type III cases but occasionally one may be required to repeat the peritoneal puncture after a lapse of four or even six hours in order to obtain a satisfactory result. In our experience a sputum in which a satisfactory typing was not obtained after six hours was either free of a fixed type of pneumococcus or contained some other organisms such as a streptococcus.

6 The mouse almost invariably survives the peritoneal puncture, but, if the organism is virulent, the mouse dies in from eighteen to twenty four hours. The peritoneum is then opened under aseptic precautions and the contents are washed out with 3 to 4 cc of saline. The washings are centrifuged, first at very low speed in order to throw down any blood cells. The supernatant fluid is drawn off and centrifuged at high speed to throw down the microorganisms.

A "ring test" or precipitin test is performed by floating about 0.1 cc of the supernatant over an equal quantity of agglutinating serum, diluted 1 in 10. At the point of contact of serum and fluid a fluffy white ring appears almost immediately in the tube containing the serum of the corresponding type. The standard macroscopic agglutination test may be made with the centrifuged sediment. The latter two tests serve as a check on the rapid method, but in our experience the "ring test" is more readily and accurately interpreted. A broth culture may be made from the heart's blood of the mouse for purposes of further examination, and usually a pure culture of the pneumococcus is obtained from this source.

In a very early case of pneumonia, the first strain isolated may not be one of the fixed types so that it is often advisable to obtain another sample of sputum and repeat the search. In this way a Type I, II, or III which otherwise would have been missed may be obtained.

TISSUE STAIN Modification of Mallory's Triple Stain, Krichesky, B. Stain Tech 6 97, 1931

Tissues fixed in Bouin's or Zenker's fixing fluids have given good results when used with this modification which is as follows:

I Staining solutions

Solution 1

Acid fuchsin (dye content 62 per cent)	0.25 gm
Water	100 cc

Solution 2

A Anilin blue	2.0 gm
Water	100 cc
B Orange G (dye content 83 per cent)	1.0 gm
Water	100 cc
C Phosphomolybdic acid 1 per cent	100 cc

Solutions A, B, and C should be kept in separate bottles because the mixture deteriorates on standing. When ready for use solution 2 is made up of equal parts A, B, and C.

II Procedure

If sections are 10 microns or less in thickness hydrate by passing through the descending series of alcohols to 70 per cent alcohol and then directly into water for five minutes. Proceed with the steps as follows:

- 1 Transfer sections to solution 1 for from one to three minutes. The time to be estimated by experience.
- 2 Wash by dipping the slide into water until the surface stain is removed.
- 3 Transfer to solution 2 for from three to five minutes or longer.

- 4 Wash, by dipping the slide into water from 1 to 3 times *only*
- 5 Transfer the slide to 70 per cent alcohol dipping hurriedly about 3 times
- 6 Transfer to 80 per cent and then to 90 per cent alcohol dipping the slide into each 5 to 8 times
- 7 Dehydrate in absolute alcohol for several minutes
- 8 Clear in xylol and mount

It has been found that two minutes is the shortest time that sections can be immersed in solution 2 for good results. If a two minute immersion is used, eliminate step 4 and proceed directly with step 5. If sections are left for a longer time in solution 2, immersion in water is necessary (step 4). Since the malin blue is readily soluble in water, the depth of this stain can be controlled by the amount of washing.

OVA, Method of Examining Urine for Helminth Eggs, Barlow, C H Am J Hyg 14 212, 1931

The necessary apparatus is simple and inexpensive. It consists of straight sided tin cans, tightly soldered and furnished with closely fitting covers, an agitating plunger, graduated cylinders on base, for measuring, pipettes for drawing off urine or a suction pump for the same purpose, centrifuge, with 50 cc and 15 cc tubes, tube rack for six 50 cc tubes, tube rack for twelve 15 cc tubes, drying cabinet or rack, slide trays for carrying slides flat, grease pencil, glass slides 38 by 75 mm in size, and a methylene blue dropping bottle.

The urine is collected in the tin cans, which are brought to the laboratory in galvanized iron boxes holding a dozen sets of cans. Each set consists of a pair of cans, one for a stool sample and one for urine. Each pair is numbered like for the convenience of the individual contributing the samples and also with a serial number in order to facilitate recording. A can holds 500 cc which is more than the average single full urination.

A common aluminum pancake turner or skimmer, which can be purchased at any five and ten cent store, is cut to fit loosely the inside of the can, and the handle is bent at right angles to the skimmer surface. This makes an agitator which thoroughly stirs the urine, but does not set up centrifugal currents but distributes the eggs evenly throughout the urine.

The urine is first measured and then poured back into the cans. It is thoroughly stirred with the agitator, held in the left hand, while it is being drawn off in a bulb pipette held in the right hand. For routine work it was found that two 50 cc tubes were needed in order to pick up eggs in light infestations and that a pipette holding just 50 cc was convenient. To prevent eggs from sticking to the sides, the pipettes must all be drawn so that there is a gradual lessening of the bore. It is essential to wash the pipettes thoroughly between each sampling by drawing up and then expelling clean water at least three times.

For known heavy or medium infestations, tubes and pipettes of 15 cc capacity are desirable, as the amount of centrifugate is decreased and the count is more easily made. Each tube of agitated urine is marked with the same legend as appears on the tin can from which it was taken.

The tubes are centrifuged at 800 rpm for one minute and then returned to the tube racks. With a cleaned pipette the supernatant urine is carefully drawn off so as not to soil the centrifugate. This may be done speedily and safely by using an ordinary suction pump attached to the sink and carrying a pipette on a rubber tube at a fixed distance from the bottom of the tubes as they stand in the rack. This obviates too constant attendance upon the tubes, and, since the suction is steadily maintained, there is no danger of roiling the centrifugate.

The remaining urine is drawn off with a small hand pipette. Just enough urine is left in the end of the tube to spread well on the slide. A drop of aqueous solution of methylene blue is added to this.

Slides 38 x 75 mm in size are prepared to receive the blued centrifugate by marking off on them, with a grease pencil, a parallelogram large enough to contain the centrifugate.

spread evenly and not too thickly. The slide should bear the same legend as the collecting can and the tube from which the centrifugate came.

The blued centrifugate in the tubes is stirred by drawing it up into a scrupulously clean pipette and ejecting it, repeating the process a few times. Care should be taken not to get bubbles of air into the pipette. All of the centrifugate is then transferred to the greased enclosure on the slide, and the slide is set aside to dry. In the Egyptian climate the drying is rapid and no special apparatus is needed. In climates which are moist any simple drying rack or oven may be used.

STAIN Differential Stain for Diagnosis of Neisserian Infection, Scudder, S. A. Stain Tech 6 99 1931

Films are made from exudate which has been skillfully selected. The exudate is collected by means of sterile cotton swabs, which are rolled (not rubbed or dragged) over clean new glass slides. The films are air dried, fixed by means of gentle heating and are stained in the following solutions. (Heat fixation gave slightly better results than fixation by means of alcohols.)

1. Flush the slide with buffered crystal violet solution three to five minutes.

Crystal violet	1 gm
Distilled water	90 cc
Buffer solution (P _H 6.6 to 7.0)	10 cc

The phosphate buffer is prepared according to the method of Clark and Lubs.

2. Decant and flush with iodine solution so that the dye iodine precipitate overflows. Time one and one half to two minutes.

Resublimed iodine	2 gm
Distilled water	90 cc
N/1 NaOH	10 cc

Solution of iodine in phosphate buffer was unsatisfactory.

2. Decant and decolorize by means of Merck's pure technical acetone, adding the acetone drop by drop until the drippings are colorless. Time ten seconds or less. Air dry.

4. Counterstain with the following differential methyl green pyronin mixture one and one half to two minutes. Prepare counterstain a day or two before using. This counterstain can be employed in examination of formalized tissue also (see Scudder and Liss, 1931).

Pyronin yellowish	0.1 gm
Over ethylated methyl green	1.25 gm
Distilled water (hot)	99 cc

Wash quickly with tap water. Air dry and examine by means of oil immersion lens and artificial daylight. Oil of bergamot is preferable to xylene for clearing the slides.

Gram positive bacteria are purple black. The majority of gram negative bacteria are faded pink, however a small influenza bacillus which has frequently been encountered in exudate strains deep magenta. The capsule of Friedlander's bacillus stains a transparent lavender.

The polynuclear nucleus is dark blue purple, the cytoplasm faint lavender. The lymphocyte nucleus is homogeneous very deep clear blue, the cytoplasm faint magenta violet to magenta. The plasma cell nucleus is dark navy blue with a cart wheel arrangement, the cytoplasm homogeneous deep magenta to purple magenta. The eosinophilic nucleus is like that of the polynuclear and granules are fairly distinct reddish and occasionally purplish. The endothelial nucleus is pale blue, the cytoplasm pinkish lavender. The red blood cell is pale magenta to bronze. Bronzing of the red blood cell is due to impregnation of dilute iodine. The nucleus of the squamous epithelial cell is purple, the cytoplasm lavender. Purplish tinting of the epithelium is due to retention of dilute crystal violet dye. The nucleus of the columnar epithelial cell of the urinary tract is more blue than that of other epithelium and

the cytoplasm stains more deeply magenta. Retention of pure methyl green gives the blue tinting and retention of dilute pyronin gives the magenta tinting. The phagocytic endothelioid cell is more reddish and more finely granular than other cells and may be found packed with gonococci in the free exudate from cases of gonorrhea.

PREGNANCY, Serum Diagnosis of, Bechuk, S. L. *Vrach Gaz.* Leningrad 3 189, 1931

The author uses the Mertz test.

To 1 cc of blood serum (hemolytic serum might be used as well) he adds 1 cc of the reagent (2 S 1000) and leaves the tubes for half an hour at room temperature. During this time the serum of the pregnant woman becomes turbid. One might add after half an hour two or three drops of bromophenol as an indicator: the serum of the pregnant will turn light blue while the normal serum will remain clear. Centrifugation is not necessary. On the basis of 303 cases observed (216 women pregnant, 20 nursing, 10 healthy with delayed menses, 23 with gynecologic disorders, 19 with cancer of various pelvic organs and 15 males) he found that the reaction is positive in 90.2 per cent of all pregnant patients. The author thinks that the second part of the reaction is not of great value and with bromophenol unavailable it may be omitted. The hemolytic serum is, of course, of no use for the color part of the reaction. Mertz' reaction is of great diagnostic value in almost 90 per cent of all patients with delayed menses, although it does not explain the causes of such a delay and is undoubtedly unable to give any definite points in the differential diagnosis. The reaction is distinctly noteworthy because of its simplicity and easy application in dispensary and office practice.

Tissue, Preparation and Staining Large Bone Sections, Wagoner, G. J. *Bone & Joint Surg.* 13 325 1931

Two methods of decalcification have been selected following extensive trial with many.

Nitric Acid. A 5 per cent aqueous solution of nitric acid will adequately decalcify large bone specimens within twenty days. The acid is changed frequently, is not the positions of the sections immersed in it.

Formic citrate.—formic citrate solution consists of equal parts of (1) 50 per cent aqueous solution of formic acid, (2) 20 per cent aqueous solution of sodium citrate. When this solvent is used, the solution is changed frequently and the position of the sections altered. Decalcification is complete in from ten to fifteen days. With the use of formic solution for decalcification there is less destruction of the cellular elements of the bone and less impairment of their staining qualities than result when nitric acid is used.

The stage of decalcification is tested either by needling the specimen, or better by shaving it with a sharp knife or razor. Accurate estimation of the process of decalcification may be obtained by the daily determination of the hydrogen ion content of the solvent.

Neutralization. If decalcification has proceeded in nitric acid, the specimen is immersed in a 5 per cent aqueous solution of potassium alum for twenty-four hours. This stage is omitted if formic citrate is employed.

Washing. The decalcified bone specimen is washed with running tap water for twenty-four hours.

Dehydration. The bone is completely dehydrated by immersing for twenty-four hours in each of a number of solutions. These solutions consist of 70 per cent alcohol, 95 per cent alcohol, absolute alcohol, and three changes of ether alcohol. The ether alcohol consists of equal parts of ether and absolute alcohol. The absolute alcohol and ether used are kept water free by overlaying a stratum of anhydrous copper sulphate.

Infiltration. Infiltration is with celloidin. Although the best results have been obtained with the use of Schering's celloidin, it has been too expensive for general use. Good results are routinely obtained by the use of Du Pont's parlodion.

Throughout the entire process of infiltration great care must be taken to maintain anhydrous conditions. The celloidin must only be made from clean, thin, oven-dried celloidin.

shavings All glassware, and utensils used in the preparation of the celloidin solutions must be thoroughly dried in the hot air oven before their use The ether alcohol used as a solvent for the celloidin must be anhydrous

Five solutions of celloidin are employed These vary in viscosity from that of No 1 which is water thin to No 5 which is of the thickness of honey Each celloidin solution is kept in an air tight container

The thoroughly dehydrated bone specimen is left in the first celloidin solution for three weeks It remains in the second solution for two weeks and in each of the remaining three solutions for one week

Evaporation After the specimen has remained in the last celloidin solution for one week, it is overlaid by a volume of the thickest celloidin solution equal to approximately five times the thickness of the specimen Evaporation is now carried out very slowly and with care to avoid bubbles The imbedding or evaporating dish is kept in a cool place As the consistency of the celloidin increases, it is first freed from the walls of the container with a knife Next a large block containing the specimen is outlined, and as the consistency increases the block is gradually freed from the surrounding mass and from day to day turned on its various faces When the various faces of the celloidin block are of equal consistency and the whole has the feel of dense rubber, further hardening is carried on by immersing the block in 65 per cent alcohol If the above process is properly executed, the celloidin block will be found to be exceedingly hard and transparent Any milkiness which may be present is indicative of the presence of water and will impair the final result

BLOCKING

The hardened celloidin block is trimmed to desired shape, leaving a margin of at least five millimeters of celloidin about the tissue It is mounted on a dry fiber block in the following manner

- 1 Immerse celloidin block in 95 per cent alcohol
- 2 Immerse celloidin block in absolute alcohol
- 3 Immerse celloidin block in absolute alcohol and ether (equal parts) until the surface feels slimy
- 4 Submerge celloidin block in thick celloidin solution and transfer quickly to the fiber block
- 5 Allow to stand in air until a thick scum forms over the celloidin (ten minutes)
- 6 Trim away excess from margin of fiber block and immerse in 70 per cent alcohol for at least twelve hours before cutting

The cut sections are stained with Weigert's iron hematoxylin and counterstained with eosin according to the following technique

WEIGERT'S IRON HEMATOXYLIN

Solution A

Hematoxylin	1 gm
Alcohol 95 per cent	100 cc

Solution B

Distilled water	95 cc
Solution iron chloride	4 cc
Hydrochloric acid	1 cc

Mix equal parts of Solutions A and B each time it is to be used

From the 70 per cent alcohol in which the sections were placed when cut, the staining process continues by immersing in

- 1 Water, to remove alcohol
- 2 Hematoxylin five minutes
- 3 Water to remove excess hematoxylin
- 4 Acid alcohol until celloidin clears

- 5 Water, to remove acid alcohol
- 6 Ammonia water, five to thirty minutes
- 7 Water, dip
- 8 Eosin, dip
- 9 Water, to remove excess eosin
- 10 Alcohol 70 per cent, one minute
- 11 Alcohol 95 per cent, until celloidin clears
- 12 Creosote

Mount from the creosote in Canada balsam on clean slides and cover. The mounted section is laid on a smooth surface and a heavy weight placed on the cover slip. This weight is allowed to remain in place for two to three days until the balsam is well set. Such a procedure aids in pressing out any wrinkles in the section and in freeing it from inclusions of air bubbles.

Lantern Slides Sections of 25 microns' thickness may be stained and mounted in balsam between the two glass plates of the ordinary lantern slide. The slide is then placed in the oven used in the routine paraffin technique and allowed to remain for from three to four days. At the end of this time the balsam will be quite firm and the slide is bound and the mat applied. There results from this procedure an excellent colored lantern slide of the desired section.

Colloidal Gold Preparation of, Patterson, J Brit J Exper Med 12 143, 1931

Reagents

- 1 Distilled water
- 2 Potassium oxalate (of analytical purity) 1 per cent solution
- 3 Gold sodium chloride (double salt), $\text{AuCl}_3 \cdot \text{NaCl} \cdot 2\text{H}_2\text{O}$
- 4 N/50 sodium hydroxide
- 5 N/50 hydrochloric acid

Notes on reagents The water is prepared by distilling ordinary tap or distilled water through an all glass (hard or resistance glass) apparatus rejecting the first 5 per cent, leaving a similar amount as residue and collecting the mid fraction for use. Although this glass distilled water has been used in the standard preparation, a gold sol nearly equal in quality results from the substitution of the ordinary laboratory distilled water furnished by the usual type of large copper still.

The oxalate is dissolved in ordinary distilled water, and is used as long as it is perfectly clear. In time a haze or slight precipitate develops when a fresh solution is made up. For the gold chloride the sodium double salt has been used as the standard reagent, but the acid salt functions equally well, the necessary adjustment corresponding to the change of gold being made apparent in the preliminary test.

The alkali is the ordinary N/50 standard solution prepared from a stock CO free sodium hydroxide, and preserved in a hard glass bottle.

THE METHOD

Preliminary Test To 50 cc of the distilled water 0.5 cc of 1 per cent potassium oxalate and 0.5 cc of gold chloride solution are added. A row of test tubes is set up and 5 cc of the mixture placed in each. The first of the series being left as made up, 1 drop of N/50 alkali is added to the second, 2 drops to the third and so on to the sixth, a standard dropping pipette being employed (one giving 25 drops to the cc). The whole series of tubes is then immediately immersed in about 70 cc of water at air temperature contained in a 250 cc hard glass beaker. The temperature of the water bath is then rapidly raised, heating being by means of a strong Bunsen burner under a thin platinum wire gauze (i.e., without an asbestos center). When the bath has reached boiling point and remained there for about 1 minute, the several tubes are withdrawn and placed in order in a rack. Of these tubes only one is representative of the right conditions for the preparation of the

stock gold sol. It is the lowest in the series to give a bright red clear solution which, when carefully examined in a good light, exhibits just the faintest sheen. Those containing less alkali eliminate themselves as exhibiting a very marked sheen, while those containing more alkali than the selected tube are too clear and purple, the total color development being also less intense. In general, when the sodium gold chloride double salt has been used tube 4 is representative of the correct conditions. It is equivalent to the use of 1 cc of gold chloride (1 per cent solution), 1 cc of 1 per cent potassium oxalate and 2.4 cc of N/50 sodium hydroxide with each 100 cc of distilled water.

The way is now open to the preparation of the gold solution itself in bulk.

Actual Preparation of the Stock Gold Solution. To 200 cc of distilled water contained in a hard glass beaker (just previously treated with aqua regia, and washed with successive quantities of tap water and distilled water) 2 cc of 1 per cent potassium oxalate, 2 cc of 1 per cent gold chloride and 4.8 cc (or such amount as has been ascertained in the preliminary test) of N/50 alkali are added. The whole is raised rapidly to the boiling point. The initial change in the formation of the hydrosol is indicated by the appearance of a light blue color, which soon darkens, and then as the temperature is further raised the solution, still remaining clear, becomes more and more purple, then red, until just before the boiling point is reached a rapid final change to a light cherry red sets in, the sol then remaining perfectly stable in color after the boiling point is attained. Once this final stage is reached the heat is withdrawn, the beaker covered and allowed to cool. When sufficiently cool it is transferred to a glass stoppered stock bottle.

Titration of Stock Gold Solution. A few drops of blood are taken directly from the finger into about 10 cc of normal saline, the red corpuscles spun down and after the supernatant liquid is removed, are washed twice with 10 cc of saline. A 1 in 100 dilution of the corpuscles is made in water, and is then used upon the gold solution exactly as a specimen of cerebrospinal fluid is used in the Lange test (i.e., with 2.5 cc of gold sol to each tube). The test is made against four different specimens of gold sol acidified as follows:

<i>Specimen</i>	<i>Amount of stock sol</i>	<i>Volume of acid added</i>
1	20 cc	0.35 cc N/50 HCl
2	20 cc	0.40 cc N/50 HCl
3	20 cc	0.45 cc N/50 HCl
4	20 cc	0.50 cc N/50 HCl

Readings are made after twenty-four hours.

With the smallest amount of acid it is usually found that the first five tubes of the Lange series are not affected, whereas with the greatest amount of acid all five will show complete precipitation. The correct addition of acid is the smallest quantity that will just enable the gold to be partially precipitated by the oxyhemoglobin, the tubes 1 to 5 to which attention is confined, showing a pale pink color. (The later tubes of the series, particularly 6 and 7, show precipitation at this stage and even with slightly lower additions of acid, but this precipitation is of a different type to that just mentioned, the obvious difference is that it leads to a bluish purple color instead of a pink, and need not be taken into consideration for the purpose of defining the end point of the titration.) In effect this is really a titration to $P_{H} 6.7-6.8$, the isoelectric point of oxyhemoglobin, which P_H refers, however, not to the gold sol itself, but to the mixture when all the reacting substances are together.

With very little experience it is possible to ascertain the correct acid addition by noting the appearance of the tubes after ten minutes, without waiting for the final reading at twenty-four hours, and also to cut down the number of specimens of acidified gold to be tested to two. It is essential to use a freshly prepared solution of oxyhemoglobin for the titration.

Immediately before use the stock gold sol is acidified in accordance with the above titration and is then completely standardized fulfilling all the requirements of the Lange reaction.

PREGNANCY, Rapid Method for Diagnosis of, Eberson, F and Silverberg, M H J A M A 96 2176, 1931

From 6 to 8 ounces (180 to 235 cc) of morning urine was obtained and 180 cc used in the test, as follows

Two and one half volumes of 95 per cent alcohol were added to the urine, and the mixture was kept in an ice chest at a temperature of from 2 to 4° C for several hours or overnight to allow the precipitate to settle out. In order to hasten the preliminary procedures, frequently the mixture was centrifugated and the sediment treated as follows. The precipitate, after centrifugation, was washed several times with from 10 to 15 cc of ether and dried in the incubator at from 37.5 to 38° C or by means of a stream of compressed air. A more satisfactory procedure was adopted by adding the ether to a suspension of the sediment in 6 cc of physiologic solution of sodium chloride shaking the mixture thoroughly, centrifugating it to remove the supernatant ether, and repeating the extraction two or three times. The supernatant saline solution after centrifugating and containing the specific hormone, now freed from the estrous or ovarian hormone, was used for injection into rats.

Female immature rats from eighteen to twenty one days old, taken from one litter, were used in the test. One cubic centimeter of the extract was injected into each of two rats on three successive days. On the third or fourth day, the animals, including an untreated normal control, were killed. The reproductive tract was examined in situ, and special attention was given to the appearance of these organs. Serial sections were prepared from the ovaries, tubes, and uterus for microscopic confirmation of gross conditions.

The technique has been improved recently to the extent of injecting 1 cc of the test material twice a day for one or two days, thus shortening the time required for diagnosis to from thirty six to forty eight hours, instead of from ninety six to one hundred hours. By this method the precipitate obtained from the urine is suspended in 3 cc of salt solution and the more highly concentrated material used for injection.

Economy in the use of alcohol for this test could be practiced by distilling the residual urine alcohol mixture and using the alcohol repeatedly. In our experience there have been no false results in numerous instances in which such reclaimed alcohol served as the reagent.

With the technique outlined there has been no mortality among the rats in the course of injections prior to the death of the animals.

INTERPRETATION OF TEST

The gross appearance of the ovaries, tubes and uterus was carefully noted. The organs, in positive tests, were uniformly enlarged, and the tubes, in particular, were distended and translucent in appearance, owing to the contained fluid. The ovaries were scrutinized for hemorrhagic protruding follicles and "blood points." In the positive instances the ovaries were unmistakably enlarged, hemorrhagic and congested. The ovarian and tubal blood vessels were engorged and stood out in bold relief as compared with normal controls. The uterus was invariably hypertrophied and turbid. In mildly positive cases seen from time to time in very early pregnancies, the gross picture was convincing despite the less pronounced changes.

Microscopically, the diagnostic criteria were the enlarged hemorrhagic follicles containing corpora lutea. The degree of luteinization varied from slight invasion at the periphery to complete transformation filling the entire structure. As will be noted subsequently, the extent of these specific changes and their progressive or retrogressive aspects were modified by the age of the fetus and certain other clinical factors. In the absence of grossly positive signs, the microscopic picture always determined the diagnosis. The presence of at least one corpus luteum was required for a positive diagnosis.

TISSUE, Rapid and Permanent Stain for Myelin Sheaths, Courville, C and Krajian, A Arch Path 11 920, 1931

Hardening For rapid diagnosis on fresh tissue, boil the blocks for one minute in 10 per cent formaldehyde and then leave in the same solution in a paraffin oven for five min

utes before cutting frozen sections When the section is not demanded in a short time, fix the tissues in 10 per cent formaldehyde for twenty four hours or longer

Sections Cut frozen sections 10 microns thick Receive sections in a large dish containing tap water

Mounting Draw sections on to slides and let the excess water drain off for about a minute and dehydrate with absolute alcohol, pouring on a few drops two or three times, each time allowing it to evaporate Blot and dip the slide in a thin celloidin fixing the section to the slide Dip the slide in tap water for a few seconds

Mordanting Cover the section with 15 per cent hot aqueous solution of ferric chloride (Ferric chloride solution should be made up fresh each time Dissolve 15 gm of ferric chloride in 100 cc of hot tap water and raise the temperature to 60° C just before use) for five minutes Drain the solution from the slide Do not wash

Staining Cover section for five minutes with equal parts of hematoxylin solution (Hematoxylin solution is prepared by dissolving 10 gm of hematoxylin crystals in 90 cc of absolute alcohol and ripening in an incubator at 37° C for two or three weeks This solution is stable and will keep for months) and distilled water, heated to 60° C This stains the section extremely black

Washing Wash thoroughly in tap water

Destaining Remove excess stain in a 1 per cent aqueous solution of ferric chloride, dipping the slide in and out of the solution until the gray matter begins to appear in contrast to the dark medullary substance This takes place in from ten to twenty five seconds

Washing Wash the slide quickly in tap water

Differentiation Cover the section with a 0.25 per cent aqueous solution of potassium permanganate and shake with the fingers to secure even differentiation which takes place in about five seconds This step should be controlled under the microscope

Washing Thoroughly wash the section in tap water

Dehydration Remove water from the section after draining off the excess by pouring on a few drops of absolute alcohol and draining off, repeating three or four times Blot between filter paper

Clearing Plunge section in a container of equal parts of aniline oil and xylene for about three minutes and then in xylene for another three minutes

Mounting Mount in Canada balsam or gum damar

BLOOD CHLORIDES Determination of Using Palladious as Indicator, Lewis, R C, and Binkley, N L Am J Clin Path 1 231, 1931

SOLUTIONS REQUIRED

- 1 Sulphosahydric acid A 2 per cent solution
- 2 Standard silver nitrate solution (1 cc 125 mg NaCl)

Silver nitrate, c p	372 grams
Nitric acid, conc	250 cc
Water to make	1000 cc

3 Standard potassium iodide solution (2 cc to 1 cc Standard AgNO_3) Transfer approximately 10 gm of potassium iodide to a liter volumetric flask dissolve in water, and dilute to volume Measure 1 cc of the standard silver nitrate solution, 0 cc of water, and 0.2 cc of palladious nitrate indicator into a small Erlenmeyer flask and titrate from a micro burette with the potassium iodide solution to the first permanent brown color After checking the titration adjust the solution by dilution so that exactly 2.03 cc instead of 2 cc of the potassium iodide solution will be required to titrate 1 cc of the standard silver nitrate solution to the brown end point with palladious nitrate indicator The extra 0.03 cc of potassium iodide solution is necessary to provide for the blank required to produce the end point with the indicator When this standard potassium iodide solution is used in the titration of excess silver nitrate in blood chloride determinations the same blank of 0.03 cc is subtracted from the titration value obtained

4 Palladious nitrate indicator solution

Palladious nitrate	0.13 gram
Nitric acid, conc	16 cc
Water to make	100 cc

This solution keeps indefinitely

TECHNIC OF METHOD

To 2 cc of plasma or whole blood in a 25 cc volumetric flask add about 6 cc of water, and then 15 cc of 2 per cent sulphosalicic acid. Dilute to volume, shake, allow to stand five to ten minutes, and filter. To 10 cc of the water clear filtrate in a 25 cc volumetric flask, add 5 cc of standard silver nitrate solution, and dilute to volume. Add a small pinch of kaolin to aid the coagulation of the silver chloride formed, shake thoroughly, allow to stand five to ten minutes, and filter. If the first few drops of the filtrate are cloudy, pour back through the filter to obtain a clear solution. To 10 cc of the filtrate in a small Erlenmeyer flask, add 0.2 cc of palladious nitrate indicator and, using a micro burette, titrate with standard potassium iodide to the first brown color. The end point is very distinct and permanent.

CALCULATION OF RESULTS

The amount of sodium chloride present may be calculated from the following formula

$$\begin{aligned} \text{Milligram of NaCl per 100 cc of blood (or plasma)} &= 2 - \frac{\text{KI used} - \text{titration blank}}{2} \\ &\times \text{mg of NaCl per cc of AgNO}_3 \times \frac{100}{\text{Blood (or plasma) equivalent of filtrate used}} \\ &= \frac{4 - (\text{KI used} - 0.03)}{2} \times 1.28 \times \frac{100}{0.32} = 4 - (\text{KI used} - 0.03) \times 200 \end{aligned}$$

Thus, to find the number of milligrams of sodium chloride in 100 cc of plasma (or whole blood), subtract 0.03 (the titration blank) from the number of cubic centimeters of KI used then subtract this figure from 4, and multiply the difference obtained by 200.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building,
Richmond, Va

*The Candiru**

HERE is a story, a medical story, that will make your blood run cold just as it did when you were a child and your negro mammy told you all about raw beef and bloody bones. When I was a child reading geography I thought how terrible it would be to be swimming in the Amazon River and have a boa constrictor drop down upon me from the trees. It seems the natives have a terror of swimming in the river but not from boa constrictors. Instead it is a very harmless looking little fish about one or two inches long called the Candiru. Unfortunately this animal is urinophilous and is strongly attracted to urine when voided in the river. Indeed, it is so strongly attracted that it will penetrate the urinary meatus. Then, of course trouble begins.

These stories of the Candiru have been more or less legendary and there have been few investigators who have actually seen the event.

Gudger who is in the department of Ichthyology in the American Museum of Natural History has made a very complete and very interesting study of the investigations that have been made in an attempt to substantiate or refute the story of the Candiru. His conclusions appear to be justified by the evidence that he presents.

An Index to the Chemical Action of Microorganisms†

THIS is a reference index which should be of great service to the bacteriologist and the chemist. It consists of three cross index tables. The first index lists the microorganisms that have been studied. Under each microorganism is listed those substances which have been used as substrates and in the next column there appear the chemical substances that are produced as a result of the action of the microorganism on the substrate. The final column gives the reference to the authors who did the experimental work. Thus, *Aspergillus niger* grown on citric acid produces glycolic acid according to the report of Challenger, Subramaniam and Walker, in 1927. The same organism grown on oxalic acid also produces glycolic acid according to the same authors. Grown on sucrose it produces citric acid according to Currie, 1917.

The second table tells the reader what may be produced on a certain substrate with specific organisms. Thus, acetic acid may be used for the preparation of formaldehyde and

*The Candiru. The Only Vertebrate Parasite of Man. By Eugene Willis Gudger, Ph.D. Bibliographer and Associate in Ichthyology, American Museum of Natural History, New York City. With a Foreword by Aldred Scott Warthin, Ph.D., M.D., LL.D., Professor of Pathology and Director of the Pathological Laboratories in the University of Michigan, Ann Arbor. With 15 Illustrations. Cloth. Pp. 120. Paul P. Hoeber Inc., New York, 1929.

†An Index to the Chemical Action of Microorganisms on the Non Nitrogenous Organic Compounds. By Ellis I. Fulmer, Ph.D., Professor of Biophysical Chemistry, Iowa State College and C. H. Workman, Ph.D., Associate Professor of Bacteriology, Iowa State College, Assistant Chief in Bacteriology, Iowa Agricultural Experiment Station. Assisted by Arthur Webb and Calvin R. Pruden, Instructors in Chemistry, Iowa State College. Cloth. Pp. 165. Charles C. Thomas, Springfield, Illinois, 1930.

NOTE. In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion culled from the volume reviewed and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in the pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

formic acid by the growth of bacillus pyocyanus according to Submiewski, 1913 Acetic acid is a substrate for the production of glycolic acid as a result of the growth of aspergillus niger according to Challenger, Subramaniam and Walker, 1927 Acetone as a substrate will produce acetic acid and formic acid following the growth of bacillus pyocyanus according to Submiewski, 1923

The third table again cross indexes, carrying the product as the major index Dextrin may be produced from starch by B amylobacter, according to Villiers, 1891 Glycerol is produced from dextrose by the action of yeast (Oppenheimer, 1913) The final table consists of the references employed in the first three

This volume should have a very definite value to research students interested in this field

*The Use of the Microscope**

THIS is a little out of the usual line It is in no sense an elementary book but is of particular interest to those who have used the microscope for a long time and who feel that they are well acquainted with all of its idiosyncrasies The average microscopist probably only obtains about 50 per cent of efficiency in magnification and detail from the use of his microscope This volume, contributed by the cytologist of the Carnegie Institution describes in detail the common errors in the use of the condenser, the illumination, the filters and screen, cover glasses, objective, mirrors, eye pieces, etc., and details the necessary steps to obtain maximum efficiency with the microscope

For routine work one may possibly be satisfied with 50 per cent efficiency but when we are dealing with experimental investigation this will not do For example, a man who is making special studies of chromosomes and even of chromomeres must provide maximum detail in order to obtain results that are at all satisfactory

In essence the volume is a treatise on the physics of light as applied to microscopy, but we will venture to say that most of the discussion deals with minutiae which are unknown to the average microscopist For those who are interested in improving their microscopic acumen, the work may be highly recommended

X-Ray Technology†

THIS is a very advanced volume on the physics of the x ray and roentgenology, of interest to the roentgenologist who desires to obtain maximum efficiency in his work The leading roentgenologists find that they must have expert physicists in association, particularly when dealing with high voltage machines used especially in therapy The writer has prepared this volume for the roentgenologist to serve in lieu of the expert services of a physicist, where the latter is not available

The Creed of a Biologist‡

THE trouble with the attitude of present day scientific men toward orthodox religion is that their attitude is negative in character They find that they cannot believe in revealed religion, but with a few exceptions a thoroughly satisfying positive creed has not been built up in its place Dr Warthin's most inspiring essay creates a positive religion based

*The Use of the Microscope A Handbook for Routine and Research Work By John Belling Cytologist Carnegie Institution of Washington First Edition Cloth Pages 315 McGraw-Hill Book Company Inc New York 1930

†X-Ray Technology The Production Measurement and Applications of X-Rays By H M Terrill Ph D Associate in Physics Institute of Cancer Research and C T Ulrey Ph D Research Physicist Westinghouse Lamp Company Cloth Pages 256 New York D Van Nostrand Company Inc 1930

‡The Creed of a Biologist A Biologic Philosophy of Life By Aldred Scott Warthin Ph D M D LL D Professor of Pathology and Director of the Pathological Laboratories in the University of Michigan Ann Arbor Cloth Pages 60 Paul B Hoeber Inc New York 1929

upon the scientific knowledge of today. It is, of course, that which most intellectual thinkers have accepted, namely the submersion of any idea or hope for personal immortality in the recognition of racial immortality. Dr. Warthin has built up a series of rules for conduct based on his religious interpretations. Race betterment through eugenic means naturally becomes a prominent factor.

The essay is most stimulating.

Bedside Interpretation of Laboratory Findings

THIS is the type of thing the general man wants. Having received such and such a report from the laboratory, how to interpret it? Dr. Wohl has done a good job in a relatively small space. His interpretations are brief, to the point and indicate an acquaintance with the current literature right up to the time of publication.

No two people would write such a book alike. Some would include more, others less. But the volume under review appears to fulfill the promise of the title very satisfactorily.

Sometimes the writer includes more than the title requires. Thus, treatment is often included. This is unobjectionable, but sometimes when one gets into the phase of treatment one finds it necessary to devote much more space than is available, in order to give it its due. For example, the author takes up desensitization treatment in allergy. This being a sideline from the main purpose of the book it is very brief and could not be followed as described without occasional risk of reaction.

A general practitioner who has received a formal laboratory report and who has some hesitancy in asking what he feels may be too simple questions will be able to dig out the answer for himself without trouble with the aid of this volume.

Practical Physiological Chemistry

THE fourth edition of Zinsser's standard work requires no introduction. Developments have been so rapid in the field of immunology and viewpoints have changed so rapidly that a book rapidly becomes out of date. As the author remarks in his preface, "In the subject of immunology seven or eight years suffice to render a young book decrepit. One

The general construction of the volume remains, as formerly, that of a practical laboratory and reference manual designed primarily as a textbook for undergraduates in physiological chemistry and serving at the same time as a reference volume for graduates.

Resistance to Infectious Diseases

THE fourth edition of Zinsser's standard work requires no introduction. Developments have been so rapid in the field of immunology and viewpoints have changed so rapidly that a book rapidly becomes out of date. As the author remarks in his preface, "In the subject of immunology seven or eight years suffice to render a young book decrepit. One

**Bedside Interpretation of Laboratory Findings*. By Michael G. Wohl, M.D., Associate Professor of Experimental Medicine, Temple University Medical School. Introduction by Joseph McFarland, M.D., Sc.D., Professor of Pathology, University of Pennsylvania. Illustrated. Cloth. Pp. 321. St. Louis: The C. V. Mosby Co. 1931.

**Practical Physiological Chemistry*. A Book Designed for Use in Courses in Practical Physiological Chemistry in Schools of Medicine and of Science. By Philip E. Hawk, M.S., Ph.D., President of the Food Research Laboratories, Inc., New York City, and Olga Lergel, M.S., Ph.D., Associate Professor of Physiological Chemistry in the University of Illinois. College of Medicine, Chicago. Tenth (25 anniversary) Edition. Pp. viii + 1011. With 2 full page plates of absorption spectra in color—4 additional full page color plates and 26 figures of which twelve are in colors. Cloth. Pp. 926. Philadelphia: P. Blakiston's Son and Co., Inc. 1931.

†*Resistance to Infectious Diseases*. By Hans Zinsser, M.D., Professor of Bacteriology and Immunology, Medical School, Harvard University. Fourth Edition. Completely Revised. Cloth. Pp. 671. New York: The Macmillan Company, 1931.

who has rashly undertaken the task of presenting this subject in book form is thereafter condemned to the alternatives of either the periodical labor of revision or a sense of guilty responsibility for many views and conceptions which he no longer supports in their original forms, unless he has become as relatively old as the book itself. Books on immunology should either be revised every ten years or destroyed except for a few museum copies."

Investigation in the field, in the last few years has been made with a far greater degree of experimental precision than in the earlier days of immunology and the more recent developments have been based upon more accurately controlled observations than those previously founded upon the trial and error methods of pure biology. The author states that this has necessitated comprehensive revision, and, while the fundamentals of the book remain unchanged, much of it has been rewritten, bringing it entirely up to date.

With a volume such as this, which deals with a constantly growing and changing subject, the appearance of a new edition at once means that preceding editions are there after chiefly of historical interest.

*The Principles of Bacteriology and Immunity**

THIS is a comprehensive book in two volumes, the standard British reference volume on this subject. The discussion of laboratory bacteriology and immunology and the review of the general subject of anaphylaxis, infection and resistance are both comprehensive and authoritative. This work has in advantage over most of the other presentations on this subject in that it also contains paragraphs on practical application.

It should be of distinct value to all interested in the general fields under consideration.

A Text-Book of Pathology†

IT IS refreshing to see a medical book that has had so many editions that the authors have ceased including prefaces to previous editions and have let it go at a single new preface to the fifteenth edition. In other words by now the work speaks for itself. That Delfield and Prudden will continue paramount among textbooks of pathology has been assured by the fact that as eminent a pathologist as Francis Carter Wood has charge of the revision.

It is a matter of some interest that the additions to our knowledge of pathology that have been made since the last edition appeared have been preeminently in the realm of functional pathology. These include further studies on the thyroid hormone, the correlation of parathyroid disease with osteitis fibrosa cystica, the work of Aschheim and Zondek on the pituitary secretion, the recent work on ovarian hormone, knowledge of the effect of ultra violet irradiation on ergosterol, the preparation of a potent extract of the adrenal cortex.

But while these are the outstanding recent advances, it is equally true that a text book of pathology more than a few years old is out of date even as regards morphologic pathology. Recent advances in our knowledge of cancer and the blood dyscrasias have contributed especially to this.

*The Principles of Bacteriology and Immunity. By W. W. C. Topley, M.A., M.D., M.Sc., F.R.C.P., Professor of Bacteriology and Immunology, University of London; Director of the Division of Bacteriology and Immunology, London School of Hygiene and Tropical Medicine; and G. S. Wilson, M.D., M.R.C.P., D.P.H., Reader in Bacteriology and Immunology in the University of London, London School of Hygiene and Tropical Medicine. In Two Volumes. Cloth. Pages 1300. New York: William Wood and Company, 1929.

†A Text-Book of Pathology. By Francis Delfield, M.D., LL.D., Some time Professor of the Practice of Medicine, College of Physicians and Surgeons, Columbia University, New York; and T. Mitchell Prudden, M.D., LL.D., Some time Professor of Pathology, College of Physicians and Surgeons, Columbia University, New York. Fifteenth Edition. Revised by Francis Carter Wood, M.D., Director of the Pathological Department, St. Luke's Hospital, New York. With 20 full page plates and 530 illustrations in the text in black and colors. Cloth. Pages 1339. New York: William Wood and Company, 1931.

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EDITORIAL

The Specific Therapy of Pneumonia

DESPITE extensive studies in recent years pneumonia still deserves Osler's famous description of this disease as "Captain of the Men of Death"

Any studies, therefore, which shed light upon the results of attempts at specific therapy are of great interest and well repay perusal

The report of Cecil and Plummer¹ is of importance covering as it does a careful survey of 1,161 cases of pneumococcus type I pneumonia

While the report is best studied in the original its high lights may be summarized with profit

Despite the fact that the pneumococcus type I is but rarely found in healthy throats this organism is responsible for by far the greatest number of cases of lobar pneumonia (30.9 per cent of 3,662 cases) These figures refer to adult cases as type I pneumonia appears to be rare in infants and constitutes only 10 per cent of the cases occurring between the ages of three and twelve years

¹Cecil R L and Plummer N Pneumococcus Type I Pneumonia J A M A 92, 1747
1929

On the other hand, as has frequently been commented upon, the disease is essentially a disease of the young, 61.8 per cent of the cases in the series occurring between ten and forty years of age.

Type I pneumonia is the prototype of lobar pneumonia in that the clinical history and course conform closely to the "typical" description.

The commonest complications in children were otitis media and empyema although various complications may be encountered in a small percentage of cases.

In the series reported it was noted that there was a steadily rising death rate from 1921 to 1929, namely, from 20 to 42.8 per cent. The reason for this is not apparent.

While many factors influence the death rate in pneumonia, among them type of patient, the incidence of alcoholism, the age and so on Cecil and Plummer note a direct relation between the death rate and pneumococcus bacteremia, the death rate with bacteremia being 66.7 per cent and but 22.5 per cent in the absence of bacteremia.

Of particular interest is that portion of the report dealing with specific therapy.

It is, of course, well recognized that while the experimental and clinical evidence in favor of the use of type I serum is quite convincing the statistical evidence is not equally convincing. The investigations of Cecil and Plummer begun in 1920 are, therefore, of great interest especially as they utilized not only type I serum but also the antibody solution of Huntoon and the concentrated serum of Felton.

Huntoon's antibody solution is polyvalent for types I, II and III, highly effective against type I pneumococci, less so against type II, and with but little value against virulent type III cultures.

The authors feel that while the freedom of Huntoon's solution from horse protein eliminated anaphylactic reactions and serum sickness it was no more concentrated than the original type I serum and also that while the clinical and statistical results following its use were very good, the severe thermal reactions produced were a distinct disadvantage. To this, also, may be added the fact that it is quite expensive.

Felton's concentrated serum was tested in 239 cases with excellent results and shown to be often ten times more potent than unconcentrated preparations so that this product deserves a more extensive use.

Finally, in the concluding words of the authors:

"Type I serum is no longer in the experimental stage. When administered early and in sufficient dosage the clinical results are striking. The present study demonstrates that concentrated serum possesses all the therapeutic value of the unconcentrated preparation. Furthermore, concentrated serum has a much higher potency, and a lower content of chill-producing substances and horse serum proteins which make it more easily administered, and less frequently followed by chills, serum sickness, and serum reactions."

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CLINICAL AND EXPERIMENTAL

A CRITICAL ANALYSIS OF THE LYON BILE DRAINAGE TECHNIC AS AN AID TO BACTERIOLOGIC DIAGNOSIS*

WITH A REPORT ON 105 CASES

BY RALPH W. NAUSS, M.D., MICHAEL LAKE, M.D., AND JOHN C. TORREY, PH.D.
NEW YORK, N. Y.

SINCE Meltzer in 1917¹ announced his theory regarding "The disturbances of the law of contrary innervation as a pathogenic factor in the diseases of the bile ducts and gall-bladder" and Vincent Lyon of Philadelphia in 1919² published the results of investigations along lines inspired by Meltzer's article, much interest has been manifested in the question of drainage of the gall bladder. Many clinicians and investigators have contributed to the rather considerable literature on this important topic. Two opposing schools of belief have arisen as to whether or not bile empties normally or can be induced to flow from the gall bladder out through the cystic and common ducts by the various means employed. Lyon and his numerous collaborators contend that such does occur normally at intervals and artificially when the proper technic is employed, while others, notably Sweet,³ Halpert,⁴ Dernel and Brummelkamp⁵ hold a more or less opposite view. It is not, however, the purpose of this paper to take a definite stand in this controversy, although for the purposes of discussion we are assuming that the "B" bile is of cystic origin. Our immediate interest lies rather in the possibilities which this so-called nonsurgical drainage of the bile ducts and gall bladder may have to offer in the field of applied clinical bacteriology.

Whipple⁶ appears to be the first to have published noteworthy studies centering around the bacteriology of Lyon's bile fractions. In his article, which was published in 1921, he offers data on 25 cases in which the gall blad-

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Received for publication April 14, 1931.

ders were subsequently removed and similar bacteriologic examinations of their contents and walls made. In about 50 per cent of these 25 cases, he found one or more varieties of bacteria, occurring in the preoperative duodenal bile, to be likewise present in the gall bladder bile or gall bladder tissue removed at time of operation. He, moreover, admits "In some of these (drainage biles), however, contaminations are suggested by the variety of type of organism." He regards the presence of *B. subtilis*, *Streptococcus salivarius* and *Micrococcus catarrhalis* as evidences of contamination in the duodenal cultures but considers the colon bacillus, hemolytic streptococcus and *Staphylococcus aureus* as etiologic factors, especially, if present in the "B" fraction following $MgSO_4$ instillation. He further states as his "impression from a study of these cases as well as some one hundred and fifty operative cases in which gall bladder bile and tissue from the gall bladder were cultured, that the colon bacillus is the most persistent of the bacteria found in the common duct." Rehfus and Lyon according to Whipple^c noted this type of persistent infection and advise autogenous vaccine therapy. Likewise other investigators, notably Kelly,⁷ as early as 1906 reported a high percentage of *B. coli* recovered from the gall bladder contents after operation.

Lyon and Kolmer in the former's book⁸ published in 1923, discussed in Chapter 19 the cultural methods applicable to bile specimens recovered by duodenobiliary drainage and reported on the bacteriologic findings for a series of 64 cases. They made cultures of A, B and C bile in both hormone agar and hormone broth, employing for the latter a special flask apparatus designed to reduce the chances of extraneous contamination to a minimum. Of this series approximately 45 per cent yielded sterile biles or obvious contaminations as shown by the plate cultures, 30 per cent were positive for *B. coli* and 22 per cent for *Staphylococcus aureus*. The broth cultures gave 16 per cent positives for streptococcus. They reported the recovered streptococci as generally virulent for rabbits and also an enhanced virulence for some of the *B. coli* and *Staphylococcus aureus* strains. They considered the recovery of any of the above organisms of probable etiologic significance and as suitable for specific therapy.

About the same time as Lyon's book appeared, Piersol and Bockus⁹ published an article entitled "A study of the bile obtained by nonsurgical biliary drainage, with especial reference to its bacteriology." They, however, report their bacteriologic findings in but five cases stating that "in the earlier portion of our work so much difficulty was experienced with the bacteriologic technic that the results obtained were too unreliable for permanent record." *Staphylococcus albus* or *aureus* was found most frequently with streptococcus and *B. coli* less often.

Boardman in an article¹⁰ published in 1924 presented comparative bacteriologic findings in a series of 56 cases. He believes it impossible to sterilize the mouth and stomach and employed a less elaborate technic in regard to sterilization than did Lyon. He took a swab from the tonsillar area, followed by thorough rinsing of the mouth with a 1 to 5,000 permanganate solution before having the patient swallow the duodenal tube. After extracting the stomach contents, this organ was rinsed with a 1 to 10,000 permanganate

solution until the washings returned clear. When the tube appeared to be in the duodenum, its position was confirmed by fluoroscopic examination after which a culture of the duodenal contents was made. Then sterile $MgSO_4$ solution (25-30 per cent) was introduced and the "B" and "C" bile portions collected from the drainage tube and cultured. No details are given regarding bacteriologic methods or technic.

Out of his 56 cases Boardman found that the fasting stomach contents, duodenal contents, "B" and "C" bile fractions gave growths similar to those obtained from the tonsil in from 46 to 69 per cent of cases and that the various bile samples gave cultures resembling those of either mouth or stomach in 74 to 86 per cent of cases. He concludes that no reliance can be placed on the bacteriologic findings in the various bile fractions, since only in 14 to 26 per cent of the bile cultures was there an absence of organisms similar to those found in the mouth and stomach. He gives no details as to media employed but states that "the various types of staphylococci and streptococci were the organisms most frequently found."

Prior to and since the time the duodenal tube was devised and the technic of its use perfected, numerous investigators have published results of studies concerning pathologic conditions in the bile, gall bladder wall and associated structures. A considerable number of these have recently been presented in tabular review by Branch¹¹. According to this table the highest percentage of positive bacteriologic growths (47 per cent) came from cultures of the gall bladder wall, while only 22 per cent of bile so examined gave positive results. In those cases listed separately as acute and chronic 71 per cent of the former gave positive cultures but only 32 per cent of the latter proved to be positive upon culturing. Approximately 13 per cent of gall stones cultivated gave positive results.

Finally the bacterial flora of the small intestine under both normal and pathologic conditions should be considered in the evaluation and interpretation of bacteriologic findings for the bile. Bienstock¹² in 1884 called attention to the fact that the upper part of the small intestine of man contains relatively few bacteria as compared with the lower part as well as the whole of the large intestine. This observation has been amply confirmed and our knowledge of the flora of the duodenum and lower parts of the small intestine in health and disease has been greatly extended through the use of the duodenal tube first employed for this purpose with infants by Hess¹³ in 1912 and by MacNeal and Chase¹⁴ on adults in 1913. Van der Reis¹⁵ in 1922 reported on a series of 350 cases and came to the conclusion that the obligate flora of the upper small intestine consisted of glycophillic gram-positive lanceolate diplococci, *B. acidophilus* types and occasional gram-negative bacilli of the aerogenes group. In the middle part of the small intestine larger numbers of bacteria were found with the gram positive cocci decreasing and the colon group increasing as lower levels were reached. Many other workers have contributed to our present knowledge of the normal and pathologic flora of the small intestine. For a concise and comprehensive review of such work up to 1924 reference is made to an article by Goldman¹⁶ in which she reports her own extensive observations.

The majority of investigators ascribe the paucity of bacteria in the upper small intestine to the germicidal or bacteriostatic effect of the gastric HCl acting as a barrier in the stomach (MacNeal and Chase,¹⁴ 1913, Hoefert,¹⁷ 1921, Gorke,¹⁸ 1922, Van der Reis,¹⁵ 1922) or as repeated waves of acidity normally passing through the small intestine (Arnold and Biody,¹⁹ 1927). On the other hand Rolly and Leibmeister²⁰ (1905) considered the important agent to be the healthy intact intestinal mucosa, Bogendorfer²¹ (1925) certain substances ("bacterostanus") which could be extracted from the duodenal mucosa and Lowenberg²² (1926) the secretions of the normal duodenal mucosa. Kendall, Day, Walker and Hauer²³ (1927) in a bacteriologic and chemical study of the duodenal contents obtained with a duodenal tube from 50 cases, apparently normal as far as the duodenum was concerned, reported that 52 per cent gave little or no growth in culture media. The remainder varied from very moderate growth yielding bacteria that were chemically inert except for acid production, to heavy growths suggestive of what might be anticipated for specimens from the lower levels of the small intestine. The authors do not state whether or not the percentages of HCl in the gastric sections of these cases were normal. Recently Rices, Sears and Downing²⁴ (1928) have reported on duodenal findings in 15 cases in which there was a normal or above normal percentage of gastric HCl. In 8 of these no growths were obtained from the material collected by tube and in 7 only moulds or yeasts. On the other hand, from 30 cases of achlorhydria, similarly examined on a fasting stomach, positive cultures were obtained in each case. *B. coli* from 10, streptococci from 24 (nonhemolytic 22 strains, hemolytic 7 strains), *Staphylococcus albus* or *aureus* from 9 and occasionally *Pneumococcus*, *B. alkaligenes*, yeasts and diphtheroids. These findings are, of course, in confirmation and extension of the reports of other investigators on the duodenal flora in achlorhydria, particularly as associated with pernicious anemia. Bogendorfer²¹ (1922), Gorke¹⁸ (1922), Seydelhelm²⁵ (1923), Goldman¹⁰ (1924), Julech²⁶ (1925), Olvet²⁷ (1926), Knott²⁸ (1927), Hoefert¹⁷ (1921) and Gorke¹⁸ (1922) found large numbers and varieties of bacteria in the duodenum of patients suffering from gall tract disease. Julech²⁶ (1925) states that disease of the duodenum or gall bladder seems to be a predisposing factor in the upward growth of bacteria in the small intestine but admits that gastric achylia may be one of the causes. On the other hand Raue²⁹ (1924) reports that cases of cholecystitis and cholangitis may show heavy growths of *B. coli* in the duodenal region in spite of abnormally high gastric free acid.

PURPOSE AND SCOPE OF THE STUDY

In view of the conflicting opinions relative to the significance of cultures recovered by the Lyon duodeno-biliary drainage technique from the A, B and C bile fractions and the probable employment of such cultures for specific vaccine therapy purposes it was thought worth while to carry out a critical bacteriologic study of bile specimens so obtained in the gastroenterology department of the Cornell Clinic, New York City. The majority of the specimens were obtained by one of us (M. Lake) but for some of the later material we are greatly indebted to Dr. J. H. Whaley. The 105 cases of Tables I and

II represent patients who had been referred to this department of the clinic for diagnosis, some of them being incipient and early cases of apparent involvement of the upper right abdominal quadrant in which gall bladder pathology was thought to play a rôle and others had been diagnosed as chronic cholecystitis. From the preceding discussion of the literature, it would seem important in estimating the value of the Lyon bile drainage technic as a means of bacteriologic study of gall bladder or bile duct infection to give due consideration to the factors which in themselves promote an unusual bacterial growth in the duodenum. In previous studies we believe that insufficient attention has been given to this point. We have, accordingly, grouped our cases under the several headings of normal gastric acidity, hyperacidity, hypoacidity and anacidity. We endeavored also to secure repeated bile specimens from as many cases as possible. Further, a comparative study was made in a series of 5 cases of the bacterial content of the duodenum and that of the "B" bile fraction to determine the likelihood of the contamination of the drained bile by the duodenal flora.

BACTERIOLOGIC METHODS AND DRAINAGE TECHNIC EMPLOYED

At the clinic the special bacteriologic apparatus and methods advocated by Lyon and Kolmer³ were employed. The former consisted of a Richardson culture flask which is prepared and used as follows: a suitable sized Erlenmeyer flask (300 c c capacity) is fitted with a two-hole soft rubber stopper containing inlet and outlet right angle glass tubes arranged so that the one projects somewhat farther into the flask than the other, the longer not intruding much over 1 inch. This flask is dry sterilized, the exposed ends of inlet and outlet tubes having been plugged with cotton-wool. The sterile broth to be used is then poured into this sterile flask, 100 c c being ample, and the sterile stopper replaced securely and firmly wired into position with suitable soft copper wire. As additional insurance of sterility, the filled flasks were placed in an Arnold sterilizer for ten minutes and after removal, cooling and drying, the cotton plugs in the inlet and outlet tubes were paraffined. In addition to these culture flasks, the clinicians were also kept supplied with sterile 20 c c cotton plugged test tubes for collecting 3 to 5 c c of bile for subsequent seeding on various media.

The procedure prior to attempts at drainage of bile was briefly as follows:

- 1 Patients were instructed to present themselves at the clinic at 9 A M on a twelve hour fasting stomach following a motor test-meal, having also been cautioned to brush the teeth thoroughly on this morning.
- 2 At the clinic they were directed to gargle and rinse the throat and mouth thoroughly with an antiseptic solution somewhat similar in composition to "Lavoris" (a proprietary preparation) followed by sterile water.
- 3 A freshly sterilized duodenal tube was then swallowed and permitted to pass to the greater curvature of the stomach.
- 4 The fasting gastric residuum was extracted by gravity or by syringe aspiration.
- 5 The stomach was then thoroughly washed with sterile water, antiseptic solution and sterile water until the returning fluid was clear.

6 Four ounces of sterile water were then introduced through the tube in order to supply the stomach with a fluid to be emptied into the duodenum and thus encourage gastric peristalsis to assist the tube into the duodenum

7 After securing proper placement of the tube, as shown by fluoroscope, the duodenal contents were carefully removed, observing definitely prescribed precautions, and set aside for study

8 The gall tracts were then stimulated to evacuation of their fluid contents by instilling into the duodenum through the duodenal tube 75 cc of a 33 per cent volumetric solution (16.66 parts by weight) of magnesium sulphate at a warm temperature. This solution is allowed to enter by gravity through the barrel of a syringe. The end of the tube is pinched to hold its siphonage and then attached to a drainage bottle. Suction by bulb or syringe is not applied unless the fluid does not run out under gravity siphonage (under a pressure head of 18 to 24 inches) and then only by a one ounce bulb

9 When bile begins to flow past the "window" in the tube the collecting bottle is changed and subsequent alterations in the gross appearance of the bile are followed also by changing the collecting bottle

If no evidence of bile has been noted in washings from the stomach or duodenum under (5) or (7), then a change from light colored to a darker colored more concentrated bile is claimed by Lyon and his followers to indicate gall bladder bile, otherwise designated as "B" bile. This fraction is the one which was usually submitted to us for bacteriologic study. (The "A" fraction is that which occupied the common and to some extent perhaps the cystic and hepatic ducts, whereas the "C" fraction is said to represent bile having its immediate source in the liver cells without much opportunity for subsequent changes to take place before collection)

The Richardson flask was seeded by removing the paraffined cotton plugs from the inlet and outlet tubes and connecting the more deeply projecting one with the distal end (beyond window) of the duodenal tube in the same manner as for the collecting bottles, sterile preparations having been made and similar precautions observed throughout. When thus connected twenty drops of the "B" bile fraction were allowed to enter this flask, upon completion of which the distal end of the duodenal tube was slipped off the inlet tube of the culture flask and then 3 to 5 cc of the same fraction collected by dripping it into a sterile test tube, sterile precautions being likewise observed

Upon receipt of this material at the laboratory, the seeded culture flask was immediately placed in the incubator and the specimen to be used for seeding other media in the refrigerator if not examined at once. A film from the latter was made to be gram-stained and examined microscopically. The broth used in the culture flask and also the plating nutrient agar were made according to the well-known "hormone" formula devised by Huntton³⁰ and employed by Kolmer in his work with Lyon.⁸

Our primary cultures, then, from each drainage of the "B" bile fraction as submitted from the clinic consisted of

1 One hundred c c of 1 per cent glucose hormone broth (P_H 74-76) in a Richardson culture flask seeded at the clinic at time of drainage with 20 drops of the "B" bile fraction direct from the drainage tube

2 One or more bromcresol purple lactose agar plates P_H 74 surface seeded from a 4 mm loop of the "B" bile fraction

3 One or more glucose blood agar plates (P_H 74) surface seeded from a 4 mm loop of the "B" bile fraction

4 Cooked-meat medium (P_H 74) seeded usually with 0.5 to 1 c c of the "B" bile fraction and vaseline sealed

5 Hormone 1.5 per cent agar containing 1 per cent glucose (P_H 74-76) for poured plates for quantitative bacterial counts

After eighteen to twenty-four hours' incubation at 37° C all cultures were inspected. Bromcresol purple lactose agar and glucose blood agar plates were surface seeded from the hormone broth flask, if any evidence of bacterial growth was observable in the latter. If not, the flask was returned to the incubator and examined again the following day. The surface seeded plates, if showing growth, were carefully examined for discrete colonies, of which the various types were fished and subcultured into 0.5 per cent hormone agar tubes preparatory to morphologic and differential study on sugar and other media. The anaerobic cultures (cooked meat broth tubes) were examined morphologically as soon as definite growth changes were evident using sterile Pasteur pipettes to penetrate the vaseline seals. At the same time a bromcresol purple lactose agar plate was surface seeded from each and also a stab culture made into a 0.5 per cent hormone agar tube. If gas formation was at all pronounced, a milk tube was seeded and vaseline capped for detection of *B. welchii*.

Isolated streptococcus cultures were seeded into an ox-gall bile medium (dried ox gall 5 per cent, peptone 1 per cent, and glucose 1 per cent) to test ability to grow in a bile environment. They were also planted into differential carbohydrate media, gelatin and other media necessary for identification. Selected *B. coli* strains were inoculated intraperitoneally into white mice as a test for virulence.

BACTERIOLOGIC FINDINGS IN THE "B" BILE FRACTIONS

In Table I is given a summary of the quantitative and qualitative bacteriologic findings together with other relevant data in the 109 examinations on 100 cases upon which this report is principally based. Primary subdivision is made upon the basis of the acidity found to exist in the stomach at a time prior to securing the specimen of bile through the duodenal tube. The groupings in this respect are as follows: normal hypo-, an-, hyper-acidity and unclassifiable for lack of a satisfactory gastric analysis. Thirty-nine or slightly over one third of the biles from these 100 cases gave negative results bacteriologically, no growth being obtained in any of the culture media employed. Of the remaining 61 cases, 40 gave total bacterial colony counts ranging from a few hundred upward. These latter are subdivided into two subgroups depending on whether or not the total count, on hormone agar plates was below or above 1000 colonies per c c of the undiluted

TABLE I
BACTERIOLOGIC AND CLINICAL DATA FOR BILE SPECIMENS OBTAINED BY THE LYON TECHNIC IN 100 CASES

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	NEG	<100	<1000	>1000			a Unstained	b Stained (Gram)		a Mucous and epithelial pig- ment	b G+ chains of fused cocci	a Micrococcus tetragenus	b G- spore bearing rods	a Neg b Neg	a Chronic cholecystitis	b Suggests pathology gall bladder																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																															
	10	9	4	Case	62	76	34	62	62								62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62

Normal free, 20 40, total, 40 60

TABLE I—Cont'd

ACIDITY CONSTANT	QUANTITATIVE BACTERIOLOGIC FINDINGS			DATE RECEIVED	SLN NO	MICROSCOPIC EXAMINATION		COLONY COUNT	BACTERIOLOGIC FINDINGS		CLINICAL FINDINGS	
	10	9	8			a	b		a	b	a	b
Normal free, 20 ±0, total, 40 ±0—Cont'd	10	9	8	1/20/28		a	b	62,500	a	b	a	b
						a	b		a	b	a	b
						a	b		a	b	a	b
						a	b		a	b	a	b
Normal free, 20 ±0, total, 40 ±0—Cont'd	10	9	8	11/7/28	10	a	b	250	a	b	a	b
						a	b		a	b	a	b
						a	b		a	b	a	b
						a	b		a	b	a	b
Normal free, 20 ±0, total, 40 ±0—Cont'd	10	9	8	11/16/29	10	a	b	12,500	a	b	a	b
						a	b		a	b	a	b
						a	b		a	b	a	b
						a	b		a	b	a	b
Normal free, 20 ±0, total, 40 ±0—Cont'd	10	9	8	12/11/28	17	a	b	58,500	a	b	a	b
						a	b		a	b	a	b
						a	b		a	b	a	b
						a	b		a	b	a	b
Normal free, 20 ±0, total, 40 ±0—Cont'd	10	9	8	1/1/29	17	a	b	750,000	a	b	a	b
						a	b		a	b	a	b
						a	b		a	b	a	b
						a	b		a	b	a	b

*No growth in 5 per cent bile peptone broth

TABLE I—Continued

ACIDITY GASTRIC CON- TENT	QUANTITATIVE BACTERIOLOGIC FINDINGS				DATE RECEIVED	SEX AGE	MICROSCOPIC EXAMINATION		COLONY COUNT	QUANTITATIVE BACTERIOLOGIC FINDINGS		CLINICAL FINDINGS	
	NEG	<100	<1000	>1000			a Unstained	b Stained (Gram)		a Plate isolations	b Flask isolations	a Clinical diagnosis	b Graham test
Hypacidity free, 5 or less 15, total, 15-35	10	3	4	6	2/ 1/27	F 49	a Mucus, bile stained pus cells, cocci and bacilli num		100	a Saprophytic cocci		a Cholecystitis	
			Case 1		3/26/27	F 50	a Bile salts, lipoid ma- terial, epithelium		100	b Streptococcus (alpha prime), S mitis		b Neg fluoroscopy shows visceroptosis	
		IV	13		9/ 8/27	F 50	b No bacteria		None made	a Streptococcus (alpha) bacillus		a Cholecystitis (mild)	
			13		11/30/27	F 30	Same		450	b Subtiloid type		b Suggests cholecystitis	
			47				a Cholesterol crystals			a Neg		a Cholecystitis with stones (definite attacks rare)	
					2/ 1/28	F 50	b No bacteria		150	b Atypical B coli		b Shows laminated stones	
			55				a Numerous round cells, exfoliative duodenitis			a Streptococcus (alpha prime), S equinus		a Chronic cholecystitis exfoliative duodenitis	
							b No bacteria			b Streptococcus (alpha prime), S equinus		b Suggests pathology gall bladder	
				Case 10	3/15/27	F 37	a None made		1,500	a S igneus		a Marked visceroptosis	
				26	5/26/27	F 50	b G+ pleomorph diplo- cocci, g+ tetrads			b S mitis and equinus		b Normal gall bladder	
Hypacidity free, 5 or less 15, total, 15-35							a Bile stained pus, mucus, short columnar cells, cholechoctus		5,700	a S mitis and salivarius		a Cholecystitis with stones	
							b No bacteria			b S salivarius		b Shows stones (opera- tion showed gall blad- der filled with stones)	

TABLE I—Cont'd

CON- TENT ACIDITY ASTIC	QUANTITATIVE BACTERIOLOGIC FINDINGS				DATE REFINED	SEX AGE	MICROSCOPIC EXAMINATION		COLONY COUNT	BACTERIOLOGIC FINDINGS		CLINICAL FINDINGS	
	10	<100	<1000	>1000			a Unstained	b Stained (Gram)		a Plate isolations	b Fisk isolations	a Clinical diagnosis	b Gram test
Hypotendity free, 5 or less 15, total, 15-35—Cont'd				6	6/21/27	F 12	a Bile stained epithelia, few crystals, clumped pigment	b G+ coccobacillus and large G- bac	7,000	a Large nonhemolytic G+ Staphylococcus	b S albus	a Cholecystitis b Suggests pathology gall bladder with stones	
				30	6/21/27	F 12	a "G," bile, same findings as above		4,500	a S salivarius	b S mitis		
				10	10/28/27	F 12	a True "B," bile choles- term crystals	b No bacteria	300	a S mitis	b S mitis		
				86	10/17/28	F 55	a No "B," bile, bile yol- low suggesting cystic duct obstruction	b No bacteria	2,500	a Yeast, atyp staphy- lococcus (hemolytic)	b S salivarius*	a Cholecystitis b Suggests pathology gall bladder	
				11	11/16/28	F 15	a No "B," bile, evidence of strais in yellow bile	b G+ cocci	650,000	a S fecalis, B acidi- facter	b S fecalis, streptococ- cus (gamma type)	a Cholecystitis (gall blad- der removed 3 yr be- fore showed stone in com duct)	
				107	1/25/29	F 17	a No evidence of gall bladder disease	b G+ chain cocci	6,600	a S fecalis* and sal- ivarius	b S albus	a Possible cholecystitis (marked visceropositis)	
												b Neg	

*No growth in 5 per cent bile peptone broth

TABLE I—Cont'd

ACIDITY GASTRIC CON- TENT	QUANTITATIVE BACTERIOLOGIC FINDINGS				DATE RECEIVED	SEX AGE	MICROSCOPIC EXAMINATION		COLONY COUNT	QUALITATIVE BACTERIOLOGIC FINDINGS		CLINICAL FINDINGS	
	NEG	<100	<1000	>1000			a Unstained	b Stained (Gram)		a Plate isolations	b Plank isolations	a Clinical diagnosis	b Gram test
	3	2	3	4	2/1/27	M 62	a Bile stained epithelia and pus cells, few crys- tals, clumps of cocci	b None made	350	a B paracoli	b B paracoli	a Cholecystitis (quan- tified liver enlarged)	b Suggests pathology gall bladder
			Case 2		12/7/28	M 52	a Clumps pigment, few bile stained epithelia and pus	b No bacteria	150	a S. salivarius*	b S. salivarius, B acid lactica	a Possible gall bladder disease, viscerotomi, (fluorescopy shows ad- hesions of duod.)	b None made
					4/21/29	M 51	a Evidence of stasis and infection of biliary tract	b Few g+ thick bac	500	a S. mitis	b S. albus	a Anacidity	b None made
			Case 4		2/15/27	M 53	a No "B" fraction, bile stained epithelia and pus	b G- rods, chain cocci	3,000	a B. aerogenes	b B. aerogenes, B pro- teus	a Cholecystitis	b Suggests pathology gall bladder
			44		11/16/27	F 71	a Cholest crystals, bile stained epithelia and pus cells, masses of bile pig- ment	b G+ pleomorph rods	30,000	a Atyp B coli	b Atyp B coli	a Cholelithiasis	b Suggests small stones (2 tests)
			54		1/18/28	M 55	a Giardia +++ in duod fluid and bile, many crystals	b Large g+ diploc, g- oval bac	1,000,000	a B. coli merogenes	b Chain streptococcus (nonhemolytic)	a Chr cholecystitis	b Neg
			54		10/24/28	M 55	a Stasis with stones, not much infection	b G+ short chain cocci	250,000	a B. coli communis	b G+ rods, enterococcus		
			54		3/27/29	M 55	a Same findings as above	b Med g- rods	300,000	a B. acid lactici, sipro- phytic cocci	b G- coliform bac		
			113		3/8/29	F 41	a Evidence of stasis and mild infection calcium crystals	b G+ pleomorph chain cocci	75,000	a S. fecalis	b G+ saprophytic cocci, rods and yeast cells	a Possible disease of gall bladder	b Neg

Anacidity free, 0, total, 5.10

TABLE I—Cont'd

ACIDITY ALYSTIC COY TENT	QUANTITATIVE BACTERIOLOGIC FINDINGS		DATE RECEIVED	SLAN NO	MICROSCOPIC EXAMINATION		COLONY COUNT	BACTERIOLOGIC FINDINGS		CLINICAL FINDINGS	
	NO	<100	<1000	>1000	a	b		a	b	a	b
	8	1	1	6	1/6/29	29		a Neg b S albus		a Possible disease gall bladder b Negative	
				Case 27	5/26/27	M 23		a A few contaminating coils b A few moulds		a Possible disease gall bladder, migraine, spas tic antium on fluotos copy b Neg	
				61	7/2/25	1	250,000	a B coli communis, B terogenes b B coli communis, B aerogenes		a Cholecystitis (spasm and adhesions of antrum on fluoroscopy) b Suggests pathology gall bladder	
				55	10/10/28	M 51	1,000,000	a S fecalis* b S fecalis*		a Cholelithiasis (common duct stone) b No gall bladder shadow (2 tests)	
				111	1/8/29	M 10	75,000	a S salivarius b Saprophytic cocci and yeast		a Hyperacidity, biliary migraine b None made	
				120	1/17/29	F 13	11,000	a S salivarius b Gt polymorphic cocci		a Hyperacidity, migraine b None made	

*No growth in 5 per cent bile peptone broth

Hyperacidity free, 35.50, total, 60.90

TABLE I—CONT'D

ACIDITY GASTRIC CON TENT	QUANTITATIVE BACTERIOLOGIC FINDINGS				DATE RECEIVED	SEX AGE	MICROSCOPIC EXAMINATION	COLONY COUNT	QUALITATIVE BACTERIOLOGIC FINDINGS		CLINICAL FINDINGS
	BACTERIOLOGIC FINDINGS								BACTERIOLOGIC FINDINGS		
	NEG	<100	<1000	>1000					a	b	
	10	2	1	5			a Unstained b Stained (Gram)			a Plate isolations b Flask isolations	a Clinical diagnosis b Graham test
			Case 116		3/27/29	F 26	a None made b G+ med diploc	1,000	a Nonhemol polymorph diplococci b G+ nonhemolytic cocci		No record
			Case 11		3/15/27	F 46	a None made b G+ biscuit diplococcus, g+ rods	7,000	a B aerogenes b B aerogenes		a Cholelithiasis b Numerous gallstones
			39	VII	10/ 6/27	F 49	a Cholesterol crystals, bile stained epith and pus b No bacteria	None made	a S albus b S albus		a Cholecystitis with stones
					10/ 6/27	F 49	a "G," bile (unmed fol lowing "B") b Occ g+ med chain cocci	None made	a S albus b None seeded		b Shows definite pathol ogy gall bladder with many small stones
			63		3/14/28	F 52	a No "B," portion, choles terin crystals, lipoids and bile stained mucus b G+ sausage shaped rods	650,000	a B acid lactici b B acid lactici		a Cholelithiasis (attacks of colic) b Suggests pathology gall bladder
			111		3/ 1/29	F 30	a Clumped pigment, bile stained epithelium and pus, mild infect b Occ clump g+ pleomorph cocci	500,000	a Neg b Neg		a Chronic disease of gall bladder (undiced) b Slightly suggestive of pathology gall bladder
			122		5/15/29	F 29	a No "B," bile—3 at tempts, pituit very nervous b Occasional large oval g+ cocci	13,000	a S equinus b Large g+ cocci		a Urinary b None made
	41	18	13	28							

Unclassified, no T M

Unclassified, no PM

bile The balance of 21 cases, giving total counts ranging below one hundred, were considered of minor value in this analysis and are accordingly not being reported in such detail as are the other 37 positive cases In each subgrouping where repeated examinations of bile from the same patient was made, the findings are brought together for convenience of comparison

In Table II the same data are given for cases in which cultures were made from the duodenal content before washing out (D B W) from the duodenum after washing out (D A W) and from the "B" bile The procedure in these cases was the same as already described except that after collection of the duodenal content, sterile water was introduced and withdrawn until it returned clear Then the magnesium sulphate solution was introduced and the "B" bile specimen obtained in the usual way The object of this experiment was to compare the flora of the duodenum both qualitatively and quantitatively with that of the "B" bile

In the following paragraphs, the data recorded in Tables I and II are analyzed in an attempt to determine whether the culturing of "B" bile is a dependable guide to the nature of gall bladder infections and if the bacterial types recovered may be accepted as suitable for the preparation of vaccines or if their source is so indefinite as to make their use unwarranted The findings in our series of cases will be considered from the following standpoints

1 Relation of cultural findings to clinical observations Of the total 100 different cases there were 39 in the group yielding negative cultures and of these negative cases there were 28 or 70.2 per cent in which the clinical tests indicated cholecystitis Of the 21 cases which showed fewer than 100 bacteria per c.c. in the bile there were 12 or 57.1 per cent with clinical evidence of cholecystitis Of the 40 cases in the groups yielding more than 100 bacteria per c.c. of bile there were 31 or 77.5 per cent in which the clinical findings were positive for cholecystitis or at least suggested its possibility There was accordingly no correlation between clinical evidence of gall bladder disease and quantitative bacterial findings just as high a percentage showed sterile bile in cases clinically suggestive of gall bladder diseases as showed high bacterial counts Of the 21 cases showing less than 100 bacteria per c.c. of bile there were 6 which yielded streptococcus and of these 4 resembled oral types which might have been brought down through swallowed saliva and 2 were *Streptococcus fecalis*, there were 2 biles yielding coliform organisms and 3 staphylococcus 2 of them albus and 1 citreus In the remaining nine cases in which organisms were recovered the types found were clearly saprophytic Of the group of 40 cases yielding over 100 bacteria per c.c., there were 21 yielding streptococcus cultures of one type or another Differential cultural tests (Hohman's classification) indicated that 21 of the total 27 streptococcus strains isolated were of oral origin viz 7 salivarius 7 mitis 2 equinus, 1 subreidus and 3 not definitely identified The remaining 6 conformed to intestinal types—*Streptococcus fecalis* (enterococcus) Only 5 of these strains were able to grow in the 5 per cent bile medium viz 3 *Streptococcus fecalis* and 2 *Streptococcus salivarius* No frankly hemolytic types

TABLE II
BACTERIOLOGIC FINDINGS FOR THE DUODENAL CONTENT (BEFORE AND AFTER WASHING DUODENUM) AND THE "B" BILE

ACIDITY GASTRIC CONTENTS	CASE NO	SOURCE OF MATERIAL	DATE RECEIVED	SEX AGE	MICROSCOPIC EXAMINATION	COLONY COUNT	BACTERIOLOGIC FINDINGS	CLINICAL FINDINGS
Hypo acidity	134	D B W	12/13/29	F 54	G+ diplococci, various sizes	14,400	Staphylococcus albus (hemo- lyticus)	Chronic cholecystitis, pathol- ogy of gall bladder, was to be operated
		D A W	12/13/29		G+ diplococci, yeast cells, few of each	1,440	As above	
		"B" bile	12/13/29		Dark viscid "B" fraction with few crystals, many pus cells and pigment in clumps No bacteria noted	20,000	As above	
Anaerod- ity	123	D B W	9/20/29	M 55	G+ cocci		S. agalvus, B. coli communis, B. welchii	Chronic cholecystitis or pathol- ogy in liver, negative Graham test
		D A W	9/20/29		G+ cocci		B. coli	
		"B" bile	9/20/29		Strains with stones, Gram G+ cocci, g- bacilli, spores estimated	2,500,000	B. coli communiur, about 15 many colonies as in D A W, B. welchii	
No T M	124	D B W	9/20/29	F 22	G+ diplococci in long chains	20,000	S. equinus, S. mitis, Strepto- coccus (nonhemolyticus)	Dermoglyphism, improved from drainage Negative Graham test
		D A W	9/20/29		G+ cocci as in D B W but scattered	1,000	S. equinus	
		"B" bile	9/20/29		"B" portion darker than nor- mal, g+ diplococci, medium and large	20,000	S. equinus, Strep (nonhemo- lyticus)	
No T M	128	D B W	10/18/29	F 29	G+ cocci, pleomorphic	150,000	Gram+ and g- cocci (like mouth saprophytes)	Urthemia, definite improve- ment with drainage Gra- ham test not done
		D A W	10/18/29		No bacteria noted	40,000	10 per cent as many colonies of same types as in D B W	
		"B" bile	10/18/29		"B" portion normal except darker than usual One clump g+ cocci	2,700	Diphtheroid (sub culture from flask)	
No T M	136	D B W	12/27/29	F 37	G+ cocci, medium and large, large g+ rod	0	No growth	Angioneurotic edema, im- proved with drainage, Gra- ham test not done
		D A W	12/27/29		G+ cocci, few	9,000	S. albus, S. viridans	
		"B" bile	12/27/29		Inky black "B" portion, g+ cocci few	250	S. albus, nonhemolyticus Streptococcus from broth flask	

were found. A few were alpha-prime and gamma as regards the changes on blood agar (Brown's classification) but most alpha (viridans). Fifteen cases yielded coliform bacilli and of these 12 were typical fermenters with gas in lactose and other differential carbohydrates while 3 did not split lactose or formed no gas. The *B. aerogenes* or the allied *B. lactici-acidi* were present in 10 cases and *B. coli communis* in only 3. A number of *B. coli* strains were tested for virulence by mouse inoculation but all yielded negative results. Where members of colon group were found at all the count was high.

Of the cases with over 100 bacteria per c.c. in the bile, there were five which yielded staphylococci, in pure culture in 3, and mixed with streptococci in 2. All the former were the albus type.

Correlating the above bacterial findings with clinical diagnosis we find that of the 19 cases positive for one or another type of streptococcus, there were 13 for which the clinical history and findings indicated gall bladder disease although the Graham test was not positive for all. In 3 of these, however, the bile examined was apparently not the "B" fraction. On the other hand there were 6 cases all yielding "B" biles from which similar types of streptococci were isolated for which neither the clinical history nor findings indicated gall bladder disease. This would suggest that the streptococcal types found in drained bile are more likely to have had their origin in the intestine or the bile ducts rather than the gall bladder. This view is further substantiated by the fact that the streptococcal types found were mostly oral types, such as *salivarius*, which are seldom associated with pathologic processes, or fecal types (*faecalis enterococcus*) of which the same may be said. The biles yielding *B. coli* were in a much higher percentage associated with gall bladder pathology than were those carrying streptococci. Eight of the 9 cases positive for *B. coli* or 88.8 per cent were clinically cholecystitis whereas only 46.1 per cent of the streptococcus-positive cases were so correlated. This might mean either that the finding of *B. coli* in "B" bile carries with it a greater assurance of gall bladder origin than does the streptococcus or that *B. coli* are more apt to be present in large numbers in the duodenum with gall bladder pathology, as has been claimed by Raue.²⁹ Repeated drainages, as will presently be indicated, favor the latter view. *Staphylococcus albus* is an organism of such feeble pathogenic propensities that we feel its presence in drained bile may well be considered of no significance and the same may be said of the great variety of other gram-positive coccic types which did not conform morphologically or culturally with the recognized pathogenic representatives of this group.

Finally the bile fractions submitted for culturing may be distributed among the following three groups: (a) satisfactory "B" portion, (b) doubtful "B" portion, (c) no "B" portion. This is based entirely on clinical observations at the time of drainage and, as is indicated in Table I, the number of satisfactory "B" portions was approximately double the number of the other two groups combined for the patients giving positive bacteriologic findings. The cultural results however showed no consistent and distinctive differences for these three classes.

2 RELATION OF THE CULTURAL FINDINGS TO GASTRIC ACIDITY

As has been stated the flora of the human duodenum is generally much richer, both as regards numbers and types, where gastric acidity is below normal or absent, than when conditions are normal in this respect. Accordingly if bacteria in the drained bile were derived in part or wholly from its passing through this region in the process of collection one might expect higher counts from cases of this type than from those in which gastric acidity is normal or above normal. Our findings as reported on this basis in Table I are to a certain extent consistent with this hypothesis. The combined figures for bile specimens from 35 cases with gastric anaacidity or hypoacidity yielded biles in which the bacterial count per c.c. was above 100 numbered 17 or 48.6 per cent whereas those from 44 cases with normal gastric acidity or hyperacidity gave counts per c.c. of bile above 100 in 34.1 per cent. In the former group streptococcal types of possible oral origin (*salivarius*, *mitis*, *ignavus*, etc.) were also somewhat more frequently encountered than in the latter. 11 times in 17 cases as contrasted with 6 times in 15 cases in the normal or above normal gastric acidity group. In the experience of one of us (J.C.T.) and that of others these are the types which are frequently found in material from the duodenum in connection with achylia gastrica.

3 CULTURAL FINDINGS IN CASES REPEATEDLY EXAMINED

There are six cases recorded in Table I, in which more than one specimen of "B" bile was examined bacteriologically and at various time intervals. Logically, if the bacteria in the bile are those causing the cholecystitis one might expect that the cultural results would be consistent with a specific infection. This however, was by no means always the case. In group I, for instance, the first drainage yielded a pure culture of *B. coli communis* but the second taken fifty-two days later a pure growth of *B. lactis aerogenes*. Group II in the first drainage showed *Streptococcus fecalis*, but in the second, performed nine days later, only *Streptococcus mitis* was isolated from both the plates and the culture flask. Group III consistently on both occasions yielded *B. lactis acidii*. Group V in drainages performed four months apart yielded entirely different types of streptococci and group VI different types of *B. coli*. Battle³¹ explains such diversity in findings from repeated examinations of a single case as due to a multiple infection of the gall bladder, but it would seem quite as logical to ascribe them to changes in the bacterial types predominant from time to time in the duodenum and jejunum and hence certain bacterial types might be picked up during the drainage process at one time and others at another. These repeated drainages yielded one quite consistent finding and that of a quantitative nature. If high bacterial counts were obtained in one examination the same was likely to occur at subsequent examinations.

4 CULTURAL FINDINGS FOR THE DUODENUM AND THE HOMOLOGOUS "B" BILE

The data assembled in Table II demonstrates clearly that one is not justified in assuming that the bacterial types isolated from the "B" bile were derived from the gall bladder. In these five cases, as stated in a preceding

paragraph, samples from the duodenum before and after washing that region were obtained and then one of the "B" bile. Two of the patients showed sub-normal gastric acidity, while for the other three no gastric analyses were made. In Case 134 a *Staphylococcus albus* in high count was the only finding for all three specimens. In Case 123, *B. coli* and *B. welchii* were recovered from the duodenal content and the "B" bile. The only difference in the findings lay in the fact that the *B. coli* of the duodenum conformed culturally to *communis* whereas that of the bile to *communior*. It seems probable that if a considerable number of colon colonies had been fished and identified that both of these types might have been found in each specimen. In Case 124 we have almost exactly the same findings for the duodenal content and the "B" bile, *Streptococcus equinus* and another nonhemolytic streptococcus of identical type in each. Case 128 yielded gram-positive and gram-negative unidentified cocci for both the specimens of duodenal content. In the "B" bile these were not found, but from it a diphtheroid-like organism was isolated. Case 136 yielded no growth from the duodenal content before washing, a *Staphylococcus albus* and a viridans streptococcus of indefinite nature from the "B" bile. In three of these cases the findings in the "B" bile were thus practically the same as for the duodenal content and in the other two the organisms recovered were strongly suggestive of mouth origin in all three specimens. In no instance was any organism of probable pathogenic nature isolated from the "B" bile which had not also been found in the "before washing" or "after washing" duodenal specimens. The colony counts also indicate that even after the duodenum has been washed with sterile water until the fluid is returned clear there are enough bacteria left in the region of the bile duct opening to account in many instances for the numbers and types of bacteria found in the "B" bile. In two of the patients, Cases 128 and 134 the types recovered suggest derivation from swallowed saliva while in the other three the organisms constituted the flora of the duodenal drainage field with possible accessions from lower levels through reversed peristalsis incident to the irritation caused by the presence of the duodenal tube tip.

5. COMPARISON OF "B" BILE BACTERIAL FINDINGS WITH REPORTS FOR INFECTED GALL BLADDEPS

In Table III we have assembled from recent literature data in regard to the bacterial content of the gall bladder as determined for operated cases. Our immediate purpose in tabulating these findings was to compare them as regards types of bacteria found and then respective percentages of incidence with those which are reported for "B" bile in this article and also by Lyon and Kolmer⁴ and by Bartle.²¹ First as regards sterility of the bile in patients operated upon for gall bladder disease both acute and chronic, we find that of the total 780 examinations including all reports in which 50 or more cases were investigated there was an average of 66 per cent sterile with a range of 45 per cent as reported by Rosenow to 88 per cent as found by Wilkie. In the Lyon and Kolmer series the drained "B" bile was found sterile in 45 per cent of instances in a series of 200 consecutive cases reported on by Bartle there were only 10 per cent sterile "B" biles and in our series 39.3 per cent. In

TABLE III
SUMMARY OF BACTERIOLOGIC FINDINGS FOR GALL BLADDERS AT OPERATION AS REPORTED IN THE LITERATURE

CASES			SOURCE OF SPECIMEN	BACTERIAL TYPES ISOLATED (PTR. CFNT)										UNIDENTIFIED	STERILE	
ACUTE	CHRONIC			COLI FORM	B TYPHO SUS	SPAPHY LOGOCOCCUS	STREPTO COCCUS	ENTRO COCCUS	B VIBRIO SUS	DIPHTHERIOID	B WFLORII					
Kelly	240		Bile	29.2	11.3	4.6	0.5 Pyogenes	-	-	-	-	2.5	53.0	240 presumably routine cases		
			Wall 61 cases	36.1	-	3.3	60.7	-	8.2	3.3	-	-	16.1			
Rosenow			Bile 55 cases	32.7	-	9.1	23.6	-	5.6	-	-	-	45.5			
	14	61	Stone 63 cases	17.5	3.2	-	77.8	-	-	16.0	-	-	9.5			
Brown	20	50	Wall	17.0	-	-	43.0	-	-	-	-	-	19.0	70 unselected cases		
	3	22	Wall Bile	16.0 32.0	-	12.0 8.0	20.0 20.0	8.0 4.0	-	-	-	-	4.0 32.0	25 operative cases		
Diennan		100 Mostly chronic	Bile 19+	12.0	-	4.0	2.0 Hemolytic	-	-	-	-	-	81.0	100 consecutive operative cases		
Johnson	12	86	Wall Bile 32+ Stone 50 cases	18.0 20.0	1.0 1.0	12.0 8.0	3.0 11.0	-	-	-	-	-	68.0 -	Histologic diagnosis on 100 consecutive operative cases		
			Wall 200 cultured	9.5	-	10.0	11.0	-	-	0.5	1.0	6.5	51.0	200 operative cases		
Judd, Mentzer and Parkhill		No cases operated in acute stage	98+ Bile 193 cultured	6.2	-	1.1	7.8	-	-	-	0.5	3.6	85.5	Confirmation in 25 cases		
			28+ Stone 67 cultured	3.0	-	-	9.0	-	-	-	-	-	64.2	Gallstones in 107 cases		

TABLE III—CONT'D

AUTHOR	CASES		SOURCE OF INFECTION	BACTERIAL TYPES ISOLATED (PER CENT)										UNIDENTIFIED	STREPTOCOCCUS	100 presumably routine cases
	ACUTE	CHRONIC		COLIFORM	B. TYPHO SUB	STAPHYLOCOCCUS	STREPTOCOCCUS	ENTEROCOCCUS	B. MUCOSUS	DIPHTHERIOID	B. PLEUROMORPHIC					
Illingsworth	12	83	Wall 100 cultured	220	—	60	390	—	—	—	—	0	380	—	100 presumably routine cases	
			Bile 100 cultured	210	—	30	170	—	—	—	—	0	600	—	100 presumably routine cases	
			Stone 23 cultured	130	—	87	87	—	—	—	—	0	695	—	100 presumably routine cases	
			7+ Cystic gland 15 cultured	133	—	0	200	—	—	—	—	67	600	—	100 presumably routine cases	
Walker	20	10	6+ Wall all contents	60	—	—	10	—	—	—	20	—	880	—	50 operative cases	
			Bile Sub mucosa	60	—	—	10	—	—	—	20	—	880	—	50 operative cases	
			Cystic gland	20	—	—	120	—	—	—	20	—	560	—	50 operative cases	
			20 Cystic gland	20	—	—	860	—	—	—	20	—	100	—	50 operative cases	
Branch	57	151	Wall 210 cultured	121	05	13	29	—	—	—	19	10	718	—	210 routine operative cases	
			73 cultured	95	05	13	28	—	—	—	10	05	810	—	Percentages related to total cases examined, as reported in Branch's Table II	
			Bile 210 cultured	110	20	53	21	—	—	—	21	10	713	—	Percentages related to total cases examined, as reported in Branch's Table II	
			Stone 91 cultured	170	—	110	220	—	—	—	—	—	500	—	300 consecutive operative cases	
Nickel and Budd	96	42	27+ Wall Cystic gland	170	—	110	220	—	—	—	—	—	500	—	300 consecutive operative cases	

Cultures from cystic lymph nodes approximately parallel to those from wall

this total of 364 cases in which the drained bile was examined bacteriologically there was thus an average of approximately 33 per cent which were found sterile or one-half the average reported for gall bladder bile. This would indicate, then, that, assuming all the "B" biles were derived from the gall bladder, the bacteria in at least one-third were added to the bile during the process of drainage either from above or below the drainage area in the intestine or from the hepatic ducts. This table also brings out the fact that without exception where both the gall bladder wall and bile were examined bacteriologically, the former yielded the higher percentage of positive cultures with an excess of positives for wall over bile ranging from 24 (Blanch) to 73 (Wilkie, submucosa) per cent. Illingsworth³² in commenting on his cultural findings makes the following statement which is applicable to a greater or less degree to most of the other reports included in the tables. "In relation to diagnosis the investigation here recorded is of interest as it affects the Meltzer-Lyon test, for, in view of the occurrence of uninfected bile in 60 per cent cases it seems obvious that a negative bacteriologic finding in this examination must be of no significance and in fact it does not even exclude the presence of gross gall bladder disease." To which on the basis of our findings for drained "B" bile we may add the conclusion that positive bacterial findings for this material in no way justifies the assumption that the organisms recovered came from the gall bladder. Wilkie³³ found further that the streptococci recovered from the submucosa of the gall bladder were inhibited and ultimately killed by the bile taken from the same gall bladder. Hence, he concludes, it is unreasonable to expect to find the infecting streptococcus in drained bile. He found also that a far higher percentage of streptococci could be recovered from the cystic lymph gland which drains the entire submucous and outer coats of the gall bladder than from the bile, his cases yielding 86 per cent positive findings for the former as against 4 per cent for the latter.

This Table III also reveals that 7 out of 8 of those who made qualitative bacterial studies of the gall bladder bile found *B. coli* more frequently than streptococci, but the reverse was true for the gall bladder wall. In our series of cases, however, streptococci were more frequently recovered than *B. coli* from the "B" bile with the respective percentages of positive findings of 44.2 and 28. These differences in prevalence of types in gall bladder bile and drained "B" bile further add to the uncertainty as to the origin of the organisms found in the latter.

CONCLUSIONS

1. A report is given on the bacteriologic findings for bile recovered by the Lyon Technic in 100 consecutive cases. The following conclusions are drawn.

We are unable to establish any satisfactory correspondence between bacterial findings for the drained bile and clinical tests for cholecystitis. Just as high a percentage of cases clinically suggestive of gall bladder disease yielded sterile biles as high bacterial counts.

Of the various types of bacteria recovered *B. coli* was found to be much more frequently associated with positive tests for gall bladder pathology than

were streptococci. It seemed probable that the latter at least, were frequently present as contamination of the drained bile through contact with swallowed saliva or from the flora of the duodenum. This conclusion was substantiated by the fact that frequently the types recovered could not thrive in 5 per cent bile, peptone broth.

In 6 cases in which more than one specimen of drained bile was examined at various time intervals there were 4 in which the findings were not consistent for an assumed specific infection of the gall bladder. This suggests an extra-hepatic origin of the bacteria.

In 5 cases in which the duodenal flora was determined before and after washing with sterile water and then compared with that of the "B" bile it was found that there was sufficient resemblance to warrant the conclusion that the bile might have derived its organisms either from the duodenal flora or from swallowed saliva. This applies not only to streptococci and staphylococci but also to *B. coli*.

The above facts and also a comparison of our bacteriologic findings and those of others for "B" biles with similar reports in the literature on direct culture of gall bladder bile at operation show certain inconsistencies which justify great hesitancy in making use of bacterial cultures isolated from drained bile in the preparation of autogenous vaccines.

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A SURVEY OF INTESTINAL PROTOZOA AMONG CHILDREN IN ST LOUIS*

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THIS survey was undertaken with the view of determining primarily incidence of intestinal protozoa among 362 white children in St Louis as compared with that found elsewhere, and second to ascertain if there existed differences in the incidence under varying conditions with respect to the general state of health and environment of children. The subjects were therefore taken from three distinct groups. The first group consisted of 164 inmates of two orphanages (110 in one orphanage and 54 in the other). A great majority of these children were apparently healthy individuals. Hygienic conditions in the first orphanage were however, much inferior to those in the second where particular emphasis is laid on sanitation and personal habits. The second group was represented by 156 patients of two hospitals for convalescents (51 in one hospital and 105 in the other). These children were still under periodic supervision of attending physicians, and most of them were suffering from various chronic ailments. Conditions with respect to hygiene and sanitation were up to standard in both hospitals. The third group consisted of 42 hospital bed-patients suffering from acute illnesses and these children were under constant care of physicians and nurses.

In many instances positive findings were secured on the first examination. When the findings were negative, however, the second specimen was secured but in no case was the examination made more than twice. Each sample of stool was examined several times and if cysts were not found by ordinary method Riva's modification¹ of the ether-acetic acid concentration method was used to verify negative finding. Direct smears were prepared from each sample of stool and emulsified in normal saline, Gram-iodin and 1-10000 aqueous eosin solutions respectively. The culture media such as Hogue's ovomucoid medium² and Boeck and Drbohlav's medium³ were used whenever necessary. Permanent slides were prepared of all positives by the Heidenhain non-hematoxylin method and were utilized for a final diagnosis whenever it was difficult to determine with certainty the species present in direct smears.

As shown in Table I the incidence of intestinal protozoa among children as influenced by the general state of health seemed to show no significant differences among the three groups. If the general state of health were a determining factor, lower incidence should have been found in the first group (healthy children) than in the other groups which included children whose vitality was low. We found however that the healthy children in one of the orphanages (A) showed the highest percentage of infection, while the lowest

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TABLE I
A COMPOSITE TABLE SHOWING THE INCIDENCE OF PROTOZOAN INFECTIONS OF INFANTINIL TRACT AMONG CHILDREN IN ST. LOUIS

GROUP	I (104 INMATES)				II (156 PATIENTS)				III (42 PATIENTS)		TOTAL (362)	
	A (110)		B (54)		C (51)		D (105)		BOY (27)	GIRL (15)		
INSTITUTION	BOY (78)	GIRL (32)	BOY (31)	GIRL (23)	BOY (28)	GIRL (23)	BOY (19)	GIRL (56)			BOY (213)	GIRL (119)
PROTOZOAN SPECIES												
Endameba coli	12	6	3	2	4	-	7	2	2	1	28	11
Endameba histolytica	3	-	-	-	-	-	-	-	-	-	3	0
Endolimax nana	-	1	-	-	1	-	2	3	-	-	3	4
Iodameba williamsi	-	1	1	-	2	-	-	-	2	-	3	1
Chilomastix mesnili	-	1	-	-	-	-	-	-	1	-	1	3
Giardia lamblia	17	8	6	2	6	6	7	8	3	-	39	24
Trichomonas hominis	-	-	-	-	-	-	-	-	-	-	0	0
Total	32	17	10	4	13	6	16	15	6	1	77	43
Double Infections												
G lamblia and E coli	10	3	-	-	1	2	-	-	2	-	12	7
G lamblia and C mesnili	1	1	-	-	-	-	1	-	-	-	1	1
G lamblia and E histolytica	1	-	-	-	-	-	-	-	-	-	2	0
G lamblia and I williamsi	1	-	-	-	-	-	1	-	-	-	1	0
G lamblia and E nana	1	-	-	-	-	1	-	-	-	-	1	1
E coli and E nana	1	-	-	-	1	-	1	1	1	-	4	1
E coli and C mesnili	1	1	-	-	-	-	-	-	-	-	1	1
E coli and I williamsi	1	1	-	-	-	-	-	-	-	-	1	0
E coli and E histolytica	1	-	-	-	-	-	1	-	-	-	1	0
E histolytica and E nana	-	-	-	-	-	-	1	-	-	-	1	0
E nana and I williamsi	-	1	-	-	-	-	-	-	-	-	0	1
Total	17	6	0	0	2	3	1	3	2	0	25	12
Triple Infections												
G lamblia, E coli and E nana	-	-	-	-	1	-	-	1	-	-	1	1
G lamblia, E coli and T hominis	-	-	-	-	1	-	-	-	-	-	1	0
G lamblia, E coli and C mesnili	2	1	-	-	-	-	1	-	-	-	3	1
G lamblia, E coli and E histolytica	1	-	-	-	-	-	-	-	-	-	1	0
G lamblia, E coli and I williamsi	1	-	-	-	-	-	-	-	-	-	1	0
G lamblia, C mesnili and I williamsi	1	-	-	-	-	-	-	-	-	-	1	0
G lamblia, C mesnili and E histolytica	1	-	-	-	-	-	-	-	-	-	1	0
Total	6	1	0	0	2	0	1	1	0	0	9	2
Grand total positive	55	24	10	4	17	9	21	19	8	1	111	57
Grand total negative	23	8	21	19	11	14	28	37	19	14	102	92

incidence occurred among sick children in the third group Furthermore, there was no mixed infection found in the latter group while in the former the incidence of such was surprisingly high and in seven out of nine instances *E histolytica* was found On the other hand, the second orphanage (B) where a rigid hygiene program is maintained showed a comparatively low incidence

Of the total of 362 children examined in 120 (33.09 per cent) the infection was limited to one protozoan species, in 37 (10.22 per cent) a double infection was found, while in 11 children (3.07 per cent) a triple infection was noted Among the intestinal protozoa, the incidence of *Giardia lamblia* was the highest, with the following species appearing in order of frequency, *Endameba coli*, *Chilomastix mesnili*, *Endolimax nana*, *Endameba histolytica*, *Iodameba williamsi* and *Trichomonas hominis* In the majority of mixed infections *Giardia lamblia* was associated with either one or two other protozoa, of which *Endameba coli* was found most commonly

The present survey revealed one of the highest incidences of intestinal protozoan infections among children reported in this country This may be accounted for by the geographic location of St Louis In this study the incidence of *Giardia lamblia* (27.3 per cent) was lower than that reported by Boeck⁴ in a survey of an industrial school for boys and girls (49.3 per cent), and that reported by Hill and Hill⁵ among children in Porto Rico (47.2 per cent), and was considerably higher than those of Maxey⁶ (15.7 per cent), Tansinsin⁷ (13 per cent), and others If in this study the number of examinations per child had approached that of Boeck (5.3 examinations per child) instead of being limited to two examinations, the incidence probably would have been found even higher The incidence of *Endameba coli* was significantly greater in this survey than in most of the others reported Whether *G lamblia* and *E coli* can establish themselves in the intestine of children more readily than other protozoa or whether their wider natural distributions in this locality are responsible for the greater incidence is difficult to determine from this study It may be suggested, however, that children are constantly exposed not only to the possibility of single or mixed infection, but also to auto-reinfection by same protozoan species The incidence of *Endameba histolytica* in this series was not high as compared with some of the surveys made elsewhere The scarcity of *Trichomonas hominis* in this study seemed

TABLE II

A COMPOSITE TABLE SHOWING AGE DISTRIBUTIONS OF PROTOZOAN INFECTIONS OF INTESTINAL TRACT AMONG CHILDREN IN ST LOUIS

AGE	PROTOZOA INFECTIONS	ENDAMEBA COLI	ENDOLIMAX NANA	IODAMEBA WILLIAMSII	CHILOMASTIX MESNII	GIARDIA LAMBLIA	TRICHOMONAS HOMINIS	DOUBLES INFECTIONS	TRIPLES INFECTIONS	QUADRUPLS INFECTIONS	PENTAPLES INFECTIONS
1-3	1	1	1	1	1	2	1	1	1	9	34
4-6	1	5	2	3	1	2	1	11	4	38	34
7-9	1	14	4	1	1	27	1	16	4	62	53
10-12	1	11	1	1	12	19	1	6	2	43	39
13-16	1	5	1	1	12	2	1	2	1	12	24
Total	5	36	7	4	4	62	6	37	11	163	192

to be due to coincidence rather than to the types of samples received on the method employed for its detection. As a matter of fact, a great majority of these specimens were examined within a few hours after collection and some of them immediately. Moreover, Hegner⁸ claimed that *Trichomonas hominis*, when kept at room temperature, exhibited no apparent diminution in number for the first few hours.

The ages of children in this survey ranged from one to sixteen years, and were tentatively placed into five groups: one to three, four to six, seven to nine, ten to twelve and thirteen to sixteen years old. A relative frequency of the intestinal protozoa based on age distribution is shown in Table II.

The highest incidence of intestinal protozoa was found among children of age-group seven to nine years with the following groups appearing in order of their frequency, ten to twelve, four to six, thirteen to sixteen and one to three years old. In all instances, the incidence was higher among boys (111 positive out of 213) than among girls (57 positive out of 149).

Faust⁹ believes that except for infections with *Giardia* there is a definite increase in the protozoan incidence from childhood to middle age. The present report shows a sudden fall in the incidence of all the protozoa as children became older. Thus, an apparent fall was observed in the group of children from thirteen to sixteen years old. A greater incidence among children between four and twelve years old may be explained by the fact that these children come in close personal contact with their playmates, some of whom are undoubtedly carriers of intestinal protozoa. Irrespective of the general state of health of individuals, they may become carriers, if sufficiently exposed to an infection by ingestion of cysts or trophozoites with food or drink.

As bearing on the question of pathogenicity of these protozoa an attempt was made to ascertain the prevalence of constipation or diarrhea among the infected children. Despite the fact that diarrhea has been considered by many as a cardinal symptom of intestinal protozoan infections, no correlation seemed to exist between intestinal protozoa and diarrhea in this study. Thus, even among children who harbored abundant cysts of *Endameba histolytica*

TABLE III

THE INCIDENCE OF INTESTINAL PROTOZOA AS CORRELATED WITH DAILY HABITS OF 105 CHILDREN IN GROUP II D (CONVALESCENTS)

BOWEL MOVEMENT	PROTOZOA ENDAMEBA COLI	ENDOLIMNA NANA	GIARDIA LAMBLIA	CHILONASTIX MESNILI	ENDAMEBA HISTOLYTICA GIARDIA LAMBLIA	ENDAMEBA COLI CHILONASTIX MESNILI	GIARDIA LAMBLIA ENDOLIMNA NANA	GIARDIA LAMBLIA ENDAMEBA COLI	ENDAMEBA HISTOLYTICA ENDOLIMNA NANA	ENDAMEBA COLI GIARDIA LAMBLIA ENDOLIMNA NANA	ENDAMEBA COLI GIARDIA LAMBLIA CHILONASTIX MESNILI	NEGATIVE	TOTAL
Normal	8	4	11	1	1	1	—	1	1	1	1	52	82
Constipation	1	1	4	1	—	1	1	—	—	—	—	12	22
Diarrhea	—	—	—	—	—	—	—	—	—	—	—	1	1
Total	9	5	15	2	1	2	1	2	1	1	1	65	105

there was no history of diarrhea, neither was there any diarrhea accompanying the presence of *Giardia lamblia*. This may be explained by assuming that a great majority of these children were "carriers," since in none of them was there the symptom that has been referred to by many as characterizing the protozoan infections of intestinal tract.

Of the total 105 children examined, 65 were negative for protozoa, 52 had a record of normal bowel movements, 12 were constipated, and 1 had diarrhea. Among 21 children harboring *G. lamblia*, there were 15 had a record of normal movements and 6 tended to be constipated and no cases of diarrhea. In two instances of *E. histolytica* associated with other protozoa the movements were reported to be regular. Moreover, the presence of a large number of protozoan cysts in one examination of stool does not seem to indicate the severity of infection, as there is constant fluctuation in the number of discharged cysts of intestinal protozoa from day to day as has been recently reported by me¹⁰ on *Giardia lamblia*.

SUMMARY

1 A survey of intestinal protozoa was conducted among three distinct groups of white children in St. Louis under varying conditions with respect to their general state of health and sanitary environment. Of 362 children examined, there were 164 healthy inmates of two orphanages, 156 patients from two hospitals for convalescents and 42 patients from a hospital for acute illnesses.

2 Among the healthy children in one of the orphanages (A) where personal hygiene was not sufficiently emphasized, the incidence was much higher than among sick children temporarily confined in the hospital. On the other hand, in the other orphanage (B) where a rigid hygiene was enforced, there was a very low incidence. This indicates that irrespective of the general state of health of children, sanitary and hygienic conditions play an important rôle in the transmission of the protozoan infections.

3 The age-groups showed that among children between seven and nine years, the incidence was the highest while the lowest was between one and three years. A very low incidence was observed among children between thirteen and sixteen years old indicating a fall in positive instances as children become older. The incidence was higher among boys than among girls.

4 There was no correlation between the presence of intestinal protozoa and diarrhea. As a matter of fact there was no single case of diarrhea among those positive (40) for protozoa, with one among those negative (65). This may be accounted for by the fact that a great majority of these children were carriers of various intestinal protozoa.

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BIOCHEMICAL AND PHARMACOLOGIC STUDY OF QUININE BI-SALICYLO-SALICYLATE*

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INTRODUCTION

THE salicylates and quinine compounds are among the most extensively investigated drugs, both experimentally and clinically. During the past half century or more many compounds of salicylic acid and of quinine have been introduced to the medical public, but none is of more peculiar interest than this recently produced compound of quinine and salysal (quinine bisalicylo salicylate). The nearest approach to this compound that had formerly been made is quinine salicylate, yet, the two compounds are quite different in their chemical properties and in their physiologic reactions. Perhaps, the most important difference between the two compounds is their reaction in acid media such as gastric juice. Quinine salicylate hydrolyzes in the stomach into quinine and salicylic acid, and, therefore, on digestion exhibits all the untoward reactions of free salicylic acid. Quinine bisalicylo-salicylate hydrolyzes in the stomach into quinine and salicylo salicylate (salysal). Now this salicylate compound which may be considered a salicyl ester of salicylic acid is practically insoluble in acid media such as gastric juice and hence does not liberate free salicylic acid in the stomach. It is therefore very much less irritating during the period just following ingestion than quinine salicylate. Hanzlick and Prashof showed that salicylo salicylate (salysal) passes through the stomach unchanged, and that it is only after it reaches the alkaline medium of the intestines that salicylic acid is liberated, and after that absorption slowly takes place. In other words, the alkali of the intestines hydrolyzes the salicylo salicylate into free salicylic and then converts it into sodium salicylate, which is soluble.

The chemical characteristics of quinine bisalicylo salicylate may be briefly listed as follows. It is a white, odorless, crystalline substance having a slightly bitter taste. It is practically insoluble in water, but in acid media such as gastric juice it separates into quinine and salicylo salicylate. The quinine content (expressed as quinine $\text{H}_2\text{SO}_4 \cdot 3\text{H}_2\text{O}$) is 45.1 per cent (quinine salicylate contains 78.9 per cent). The salicylic acid content is 65.8 per cent (quinine salicylate contains 28.7 per cent).

The physiologic actions of quinine and salicylate acid are known to overlap in many respects but it is evident that their similar effects are produced

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in different manners. Quinine acts as a stimulant to the nervous system, it is a slight respiratory stimulant and a depressant to the circulation, it lessens the ameboid movement of the white corpuscles, it possesses a mild antiseptic action, and it reduces fever by diminishing heat production by a peripheral depression of nitrogenous metabolism. In other words, quinine affects the general metabolism of all forms of protoplasm, and in large doses acts as a general protoplasmic poison. Salicylic acid, on the other hand, is stated to exert its beneficial therapeutic influence through its specific antiseptic effect and it reduces fever by increasing heat dissipation.

From the above remarks, it is evident that this new compound, quinine bi-salicylo salicylate, should be useful whenever combined quinine and salicylic medication is indicated, particularly in painful and febrile diseases, such as rheumatism, tonsillitis, influenza, and neuralgia.

The object of the present study was to determine the biochemical properties and pharmacologic action of this compound as compared with analogous experimental quantities or doses of quinine in the forms of quinine sulphate and quinine salicylate, and salicyl in the forms of sodium salicylate, salicylo-salicylate, and quinine salicylate, etc.

SOME CHEMICAL REACTIONS OF QUININE BI-SALICYLO SALICYLATE

1 Aqueous suspension of quinine bi-salicylo-salicylate + FeCl_3 solution (salicylic acid test) = light purple. The color intensified on standing.

2 Aqueous suspension of quinine bi-salicylo-salicylate + dil. HCl solution and heat, then neutralized and treated with FeCl_3 solution = purple color. The reaction, however, was only slightly more positive than that obtained in Exp. 1, thereby showing that dilute hydrochloric acid is not a good hydrolyzing agent for quinine bi-salicylo salicylate.

3 Aqueous suspension of quinine bi-salicylo salicylate + NaOH solution, plus heat, then neutralized and treated with FeCl_3 solution = intense purple color. It is evident that hydrolysis was good and that sodium salicylate was formed. Of course the neutralizing of the mixture liberated free salicylic acid.

4 Aqueous suspension of quinine bi-salicylo salicylate plus very dilute H_2SO_4 solution, plus heat, plus alkaloidal reagents yielded the usual alkaloidal precipitation.

5 Aqueous suspension of quinine bi-salicylo-salicylate on filtering and treating the filtrate with alkaloidal reagents gave the usual precipitate.

6 Some of the filtrate from Exp. 5 on treating with dilute H_2SO_4 became fluorescent (quinine reaction).

7 Quinine salicylate plus water, plus FeCl_3 solution gave a dark purple color immediately.

8 Acetylsalicylate plus water, plus FeCl_3 solution gave a dark purple color immediately.

9 Quinine bi-salicylo-salicylate on treatment with ether leaves a residue which gives a dark purple color on treatment with FeCl_3 solution.

10 Quinine bi-salicylo-salicylate is readily soluble in cold chloroform and in hot acidified alcohol

11 In aqueous solutions of varying P_H it was found that quinine bi-salicylo-salicylate is most soluble in alkaline solutions of about P_H 10, next in acid solutions of about P_H 2 and least soluble in solutions of P_H 7

DIFFUSION OF QUININE BI-SALICYLO-SALICYLATE THROUGH COLLODION SACS AT DIFFERENT P_H

Each sac contained 1 gram of quinine bi-salicylo-salicylate plus 20 cc of water of the varying P_H indicated below, and was allowed to diffuse into water of the same P_H

TABLE I

TIME	TESTS	P_H 1	P_H 2	P_H 3	P_H 6	P_H 7	P_H 8	P_H 10
15 min	FeCl ₃ test	-	-	-	-	-	-	-
	Alk ppt	-	-	-	-	-	-	-
30 min	FeCl ₃ test	-	-	-	-	-	-	-
	Alk ppt	-	-	-	-	-	-	-
1 hr	FeCl ₃ test	-	-	-	-	-	-	-
	Alk ppt	-	-	-	-	-	-	-
2 hr	FeCl ₃ test	±	±	±	-	-	-	±
	Alk ppt	-	-	-	-	-	-	-
3 hr	FeCl ₃ test	-	±	±	±	-	-	±
	Alk ppt	-	-	-	-	-	-	-
6 hr	FeCl ₃ test	±	±	-	-	-	±	±
	Alk ppt	±	±	±	±	±	-	±
24 hr	FeCl ₃ test	±	-	-	±	-	-	±
	Alk ppt	±	-	±	-	-	+	-

It will be noted that diffusion was greatest at P_H 10 and next at P_H 2

DIFFUSION OF QUININE BI-SALICYLO-SALICYLATE IN THE PRESENCE OF PROTEIN SOLUTION AT VARYING P_H

Each diffusion sac contained 1 gram of quinine bi-salicylo-salicylate suspended in 20 cc of water of different P_H plus 5 cc of a saturated solution of egg albumen. The diffusion was into solutions of the same P_H

TABLE II

TIME	TESTS	P_H 1	P_H 2	P_H 3	P_H 6	P_H 7	P_H 8	P_H 10
15 min	FeCl ₃ test	-	-	-	-	-	-	-
	Alk ppt	-	-	-	-	-	-	-
30 min	FeCl ₃ test	-	-	-	-	-	-	-
	Alk ppt	-	-	-	-	-	-	-
1 hr	FeCl ₃ test	-	-	-	-	-	-	-
	Alk ppt	-	-	-	-	-	-	-
2 hr	FeCl ₃ test	-	-	-	-	-	-	±
	Alk ppt	-	-	-	-	-	-	-
3 hr	FeCl ₃ test	-	-	±	-	-	±	-
	Alk ppt	-	-	-	-	-	-	-
6 hr	FeCl ₃ test	±	-	-	-	-	-	±
	Alk ppt	-	-	±	±	-	-	-
24 hr	FeCl ₃ test	-	-	±	±	-	-	-
	Alk ppt	-	-	±	±	-	-	-
48 hr	FeCl ₃ test	-	-	-	-	-	-	±
	Alk ppt	-	-	-	-	-	-	±

These results indicate that albumin does not interfere with the hydrolysis and diffusion of quinine bi-salicylo-salicylate, i.e. the results are practically the same as those obtained in the previous table

DIALYSIS OF QUININE SULPHATE PLUS SODIUM SALICYLATE FROM SOLUTIONS OF DIFFERENT P_H

Each diffusion sac contained $\frac{1}{2}$ gram of quinine sulphate plus $\frac{1}{2}$ gram of sodium salicylate dissolved in 20 cc of water of different P_H and were diffused into solutions of the same P_H

TABLE III

TIME	TESTS	P_H 1	P_H 2	P_H 3	P_H 6	P_H 7	P_H 8	P_H 10
15 min	FeCl ₃ test	-	+-	+	+	+++	+	-
	Alk ppt	-	-	-	-	-	-	-
30 min	FeCl ₃ test	-	+++	+++	++	+++	++	+
	Alk ppt	-	-	-	-	-	-	-
1 hr	FeCl ₃ test	+-	++++	++++	+++	+++	+++	+
	Alk ppt	-	-	-	-	-	-	-
2 hr	FeCl ₃ test	+	++++	++++	++++	++++	++++	++++
	Alk ppt	+	+	+	+	-	-	-
3 hr	FeCl ₃ test	++++	++++	++++	++++	++++	++++	++++
	Alk ppt	+++	++	+	+	-	-	-
6 hr	FeCl ₃ test	++++	++++	++++	++++	++++	++++	++++
	Alk ppt	++++	++++	++++	++	-	-	-
24 hr	FeCl ₃ test	++++	++++	++++	++++	++++	++++	++++
	Alk ppt	++++	++++	++++	++	-	-	-
48 hr	FeCl ₃ test	++++	++++	++++	+++	++++	++++	++++
	Alk ppt	++++	++++	++++	+++	+-	-	-

Table III presents quite a different picture from the results obtained with quinine bi salicylo salicylate. In this case P_H 7 is the reaction point at which the salicylate is most diffusible and the maximum rate is attained within thirty minutes. The quinine is diffusible in acid medium only and starts between one and two hours, the maximum rate being attained at about six hours.

DIALYSIS OF QUININE SULPHATE PLUS SODIUM SALICYLATE IN THE PRESENCE OF PROTEIN SOLUTIONS OF VARYING P_H

In each diffusion sac were placed $\frac{1}{2}$ gram of quinine sulphate, $\frac{1}{2}$ gram of sodium salicylate, 20 cc of water of a given P_H and 5 cc of saturated egg albumen solution. These were placed in water of the same P_H as that present in each sac.

TABLE IV

TIME	TESTS	P_H 1	P_H 2	P_H 3	P_H 6	P_H 7	P_H 8	P_H 10
15 min	FeCl ₃ test	-	-	-	-	-	-	-
	Alk ppt	-	-	-	-	-	-	-
30 min	FeCl ₃ test	-	+	+-	+-	++	+-	-
	Alk ppt	-	-	-	-	-	-	-
1 hr	FeCl ₃ test	+-	++	+	+	++++	+	-
	Alk ppt	-	-	-	-	-	-	-
2 hr	FeCl ₃ test	+	+++	++	+	++++	++	+-
	Alk ppt	+	+	+	-	-	-	-
3 hr	FeCl ₃ test	+	++++	+++	+	++++	+++	+
	Alk ppt	++	++	++	-	-	-	-
6 hr	FeCl ₃ test	++	++++	++++	++	++++	++++	++
	Alk ppt	++++	++++	++++	+-	-	-	-
24 hr	FeCl ₃ test	++++	++++	++++	++++	++++	++++	++++
	Alk ppt	++++	++++	++++	+	+	+	-

The results of this experiment apparently indicate that albumin has a greater retarding effect on the diffusion of quinine sulphate and sodium salicylate than it does on quinine bi salicylo salicylate.

DIALYSIS OF QUININE SULPHATE PLUS SALICYL-SALICYLATE (SALYSAL) IN
SOLUTIONS OF VARYING P_H

In each dialyzing sac were placed $\frac{1}{2}$ gram of quinine sulphate, $\frac{1}{2}$ gram of salysal and 20 cc of water of different P_H . Each sac was then placed in a solution of the same P_H .

TABLE V

TIME	TESTS	P_H 1	P_H 2	P_H 3	P_H 6	P_H 7	P_H 8	P_H 10
15 min	FeCl ₃ test	-	-	-	-	-	-	-
	Alk ppt	-	-	-	-	-	-	-
30 min	FeCl ₃ test	-	-	-	-	-	-	-
	Alk ppt	-	-	-	-	-	-	-
1 hr	FeCl ₃ test	-	-	-	-	+-	+	-
	Alk ppt	-	-	-	-	-	-	-
2 hr	FeCl ₃ test	-	-	-	-	+	+	++
	Alk ppt	+	-	-	-	-	-	-
3 hr	FeCl ₃ test	+	+	-	-	+	-	+++
	Alk ppt	++	+	+	-	-	-	-
6 hr	FeCl ₃ test	++	+	+	+	+	-	+++
	Alk ppt	+++	+	+	-	-	-	-
24 hr	FeCl ₃ test	++++	+++	++	++	++	++	++
	Alk ppt	+++	++	++	+	-	-	-

The results are quite similar to those obtained with quinine bi-salicylo-salicylate

TESTING FOR QUININE AND SALICYLATES IN ANIMAL'S BLOOD FOLLOWING THE
ADMINISTRATION OF QUININE BI-SALICYLO-SALICYLATE BY MOUTH

The following tests were in the nature of trial methods for the detection of quinine bi-salicylo-salicylate or its cleavage products after the administration of this compound to animals

1 A rabbit was given 1 gram of quinine bi-salicylo-salicylate by mouth. This was accomplished by means of a syringe and rubber tubing. About 200 cc of water were required to get all of the drug into the animal's stomach. Twenty hours later the animal was exsanguinated. After laking the blood was shaken with several times the volume of chloroform. The chloroform was evaporated to dryness on a steam-bath, then portions of the residue were tested for quinine bi-salicylo-salicylate, quinine and salicylates. All were negative.

A portion of the hemolyzed residue from the chloroform extract was then tested for salicylates as follows. The hemolyzed blood was saturated with ammonium sulphate and rendered acid to about 0.6 per cent with H_2SO_4 , brought to the boil, filtered, cooled, neutralized with NaOH solution, extracted with chloroform (4 times), evaporated and the residue from this was tested with $FeCl_3$ solution. A strong positive test for salicylates was obtained.

Another portion of the hemolyzed blood was tested for quinine according to the standard technique. The result was negative.

2 A rabbit weighing 2250 grams was given 5 grams of quinine bi-salicylo-salicylate by mouth in the manner indicated above. After eighteen hours the animal was exsanguinated and the blood tested for quinine bi-salicylo-salicylate, quinine and salicylates. Only salicylates were found. The urine likewise yielded only salicylates.

3 A large dog was given 10 grams of quinine bi-salicylo-salicylates at 2 P.M. on one day and at 11 A.M. the next day he was given another 10 grams. One half hour after receiving the second dose the animal was bled. There

were 680 c c of blood obtained. An attempt was then made to recover unchanged quinine bi-salicylo-salicylate, quinine and salicylates from this blood by means of the well-known Stas-Otto process. We did not find the method practicable with so much blood, i e, we could not get the product clear. We were obliged to finish up with ammonium sulphate acidified to about 0.6 per cent with sulphuric acid. A good test was obtained for salicylates only.

QUALITATIVE AND QUANTITATIVE ESTIMATION OF QUININE BI-SALICYLO-SALICYLATES AND ITS CLEAVAGE PRODUCTS (QUININE AND SALICYL) IN BLOOD

While performing the foregoing tests we found that the detection and estimation of quinine bi-salicylo-salicylates and its cleavage products, quinine and salicyl presented many difficulties. None of the standard methods was quite satisfactory. After many trials we finally evolved a satisfactory method. This method is a blend of several methods used in the estimation of quinine and salicyl plus some modifications of our own.*

Technic—Five c c of blood are laked with 5 c c of distilled water in a small beaker, then 40 c c of saturated ammonium sulphate containing 0.6 per cent sulphuric acid are added to it and 5 grams of solid ammonium sulphate. The mixture is then heated on a moderately warm electric hot plate with constant stirring. After coagulation takes place the liquid is brought to the boil over a free flame, and immediately filtered through a Gooch crucible. The suction is continued until the mat is quite hard. Instead of using asbestos in the Gooch crucible, a single layer of high grade filter paper is more practicable. By means of a fine-bladed knife the contents of the crucible are transferred back to the beaker. The filter paper is washed with 3 or 4 c c of water, and a new paper is placed in the crucible. The coagulum in the beaker is macerated in the water used to wash the filter paper, then 40 c c of acid, saturated ammonium sulphate are added and the mixture is heated on the electric hot plate, then brought to the boil and filtered, this process is repeated four more times, only that in last three times it is preferable to use plain saturated ammonium sulphate.

The entire filtrate (about 250 c c) is transferred to a graduated flask and made up to volume. This solution can be used for the detection and estimation of quinine bi-salicylo-salicylate or for the detection and estimation of its cleavage products, quinine and salicyl. (The estimation of all three substances in the same solution is difficult to accomplish.)

1 *Estimation of Quinine*—Prepare (1) a standard solution of quinine containing 1 part of quinine base (0.0134 gm of quinine sulphate per liter was used) per 100,000 in saturated ammonium sulphate. (2) Prepare an acidified iodine solution. This is made by diluting 25 c c of N/10 iodine solution with 225 c c of N/10 HCl.

A definite amount of this acidified N/100 iodine solution is used with different concentrations of the standard quinine solution for comparative

*The most helpful method was that entitled *Estimation of Minute Quantities of Quinine in the Blood* by A. C. Roy, *Ind. J. Med. Research* 14: 129, 1926.

purposes This test is very delicate A difference of 0.001 mg of quinine can easily be detected by means of a colorimeter

For comparison with the unknown quinine solution a series of 10 tubes are made up as shown in Table VI

TABLE VI

TUBE	QUININE SOLUTION	C.C. OF SATURATED
	C.C. OF STANDARD	AMMONIUM SULPHATE SOLUTION
1	5.0	--
2	4.5	0.5
3	4.0	1.0
4	3.5	1.5
5	3.0	2.0
6	2.5	2.5
7	2.0	3.0
8	1.5	3.5
9	1.0	4.0
10	0.5	4.5

In another tube (all the tubes should be of the same size) 5 c.c. of the ammonium sulphate extract of the blood are placed One-half c.c. of the iodine reagent is added to each tube Then, the "unknown" tube is matched against the standard tubes First roughly by means of the eye, then by means of a colorimeter with a light attachment We found the Klett colorimeter very satisfactory

It is desirable to compare the solutions immediately after mixing with the iodine solution as a turbidity develops on standing

2 *Estimation of Salicyl*—Four-fifths of the ammonium sulphate extract of the blood are placed in a separator funnel and extracted with about 30 c.c. of pure ether This process is repeated 3 or 4 times The ether extract is placed in a large test tube (10" $1\frac{1}{4}$ ") and evaporated by placing in warm water, the evaporation of the first portion of ether taking place as the second is being shaken with the ammonium sulphate extract, etc

The final residue is dissolved in 15 c.c. of warm saturated ammonium sulphate solution To 5 c.c. of this solution are added 10 drops of a freshly prepared solution of ferric ammonium sulphate (2 per cent) and then comparison is made against varying amounts of a standard solution of sodium salicylate The standard solution contains 1-10.000 of salicyl and the solvent is neutral saturated ammonium sulphate

Ten tubes containing varying amounts of the standard salicyl solution are prepared in the manner indicated for quinine and 10 drops of the ferric ammonium sulphate solution are added to each of these tubes The tube containing the unknown amount of salicyl is then compared with these tubes by means of a Klett colorimeter (or other type provided with an electric lighting system)

NOTE—The quinine may be estimated in a 5 c.c. portion of the above solution but a greater amount of the iodine solution may be required to overcome the effect of the greater concentration of salicyl

3 *Estimation of Quinine Bi-salicylo-salicylate*—Quinine bi-salicylo-salicylate can be estimated by the method described for quinine, since it also gives a definite color with the iodine reagent. The standard solution in this case being a 1-100,000 solution of quinine bi-salicylo-salicylate dissolved in saturated ammonium sulphate solution. 1 c.c. of the iodine solution is added to each tube of standard and unknown solutions.

Unhydrolyzed quinine bi-salicylo-salicylate does not give the salicyl-ferrie alum test.

RATE OF ELIMINATION OF THE CLEAVAGE PRODUCTS OF QUININE BI-SALICYLO-SALICYLATE FROM A NORMAL MAN

0.8 gram (12 grains) of quinine bi-salicylo-salicylate were taken at one dose by a normal adult man. There were 250 c.c. of water taken with the drug. The rate and duration of elimination of the cleavage products (quinine and salicyl) in the urine was then tested. The results are tabulated below.

TABLE VII

TIME	URINARY PRODUCT	TIME	URINARY PRODUCT
10 minutes	Quinine = - Salicyl = -	12 hours	Quinine = - Salicyl = + + + +
20 minutes	Quinine = - Salicyl = -	24 hours	Quinine = - Salicyl = + + +
30 minutes	Quinine = - Salicyl = + -	30 hours	Quinine = - Salicyl = + +
40 minutes	Quinine = - Salicyl = +	36 hours	Quinine = - Salicyl = +
50 minutes	Quinine = - Salicyl = +	42 hours	Quinine = - Salicyl = +
60 minutes	Quinine = - Salicyl = + +	48 hours	Quinine = - Salicyl = +
90 minutes	Quinine = + - Salicyl = + +	54 hours	Quinine = - Salicyl = +
2 hours	Quinine = + Salicyl = + + +	60 hours	Quinine = - Salicyl = + -
4 hours	Quinine = + + + + Salicyl = + + +	72 hours	Quinine = - Salicyl = -
8 hours	Quinine = + Salicyl = + + + +		

It will be noted that the salicyl could be detected an hour before the quinine fraction and that it could still be detected for many hours after negative results were obtained for quinine.

TOXICITY AND FATAL DOSES OF QUININE BI-SALICYLO-SALICYLATE AS COMPARED WITH VARIOUS QUININE AND SALICYL MIXTURES

A large number of rabbits were used in the toxicity experiments but as the details are of little moment, only the average results will be cited.

All the animals were kept on the same diet, which was a full diet of bread and vegetables.

TABLE VIII

LETHAL OR TOXIC TITERS

Dose given was quinine bisalicylate solution	
1 gm. of quinine bisalicylate solution per kilo	nontoxic
1 gm. of quinine bisalicylate solution per kilo	nontoxic
1 gm. of quinine bisalicylate solution per kilo	nontoxic
1 gm. of quinine bisalicylate solution per kilo	toxic and fatal
1 gm. of quinine bisalicylate solution per kilo	toxic and fatal
2 gm. of quinine bisalicylate solution per kilo	toxic and fatal
Quinine sulphate + sodium salicylate equivalent in quinine and salicyl content to	
1 gm. of quinine bisalicylate solution per kilo	nontoxic
1 gm. of quinine bisalicylate solution per kilo	toxic and fatal
1 gm. of quinine bisalicylate solution per kilo	toxic and fatal
2 gm. of quinine bisalicylate solution per kilo	toxic and fatal
Quinine sulphate and salicyl equivalent in quinine and salicyl content to	
1 gm. of quinine bisalicylate solution per kilo	nontoxic
1 gm. of quinine bisalicylate solution per kilo	toxic and fatal
1 gm. of quinine bisalicylate solution per kilo	toxic and fatal
1 gm. of quinine bisalicylate solution per kilo	toxic and fatal
Quinine + salicyl equivalent in quinine and salicyl content to	
1 gm. of quinine bisalicylate solution per kilo	nontoxic
1 gm. of quinine bisalicylate solution per kilo	nontoxic
1 gm. of quinine bisalicylate solution per kilo	toxic and fatal
1 gm. of quinine bisalicylate solution per kilo	toxic and fatal
Quinine salicylate + sodium salicylate equivalent in quinine and salicyl content to	
1 gm. of quinine bisalicylate solution per kilo	nontoxic
1 gm. of quinine bisalicylate solution per kilo	nontoxic
1 gm. of quinine bisalicylate solution per kilo	toxic and fatal
1 gm. of quinine bisalicylate solution per kilo	toxic and fatal

SUMMARY OF THE ABOVE RESULTS

DOSES	MAXIMAL TOLERATED DOSE GM PER KILG	MINIMUM LETHAL DOSE GM PER KILG	REMARKS
I Quinine bisalicylo salicylate	1.00	1.25	
II Quinine sulphate + sodium salicylate	0.75	1.00	Quinine and salicyl equiv. to that in quinine bisalicylo salicylate
III Quinine sulphate + salicyl	0.50	0.75	Quinine and salicyl equiv. to that in quinine bisalicylo salicylate
IV Quinine salicylate	1.00	1.25	Quinine only equiv. to that in quinine bisalicylo salicylate
V Quinine salicylate + sodium salicylate	0.75	1.00	Quinine and salicyl equiv. to that in quinine bisalicylo salicylate

It is interesting to note that the values in II and V are the same

The temperature and respiration factors were also noted in the above experiments and the following data were observed

1 In every case there was a drop in temperature following the administration of the drug, but no proportionality between the amount of drop and the dosage could be observed

2 The respiration was very slightly affected with nontoxic doses. With toxic doses the respiration exhibited a marked increase. When lethal doses

were given the respiration markedly increased for several hours before death, followed by a decrease just before death

THE ABSORPTION OF QUININE BI-SALICYLO-SALICYLATE AND ALLIED SUBSTANCES FROM THE STOMACH AND INTESTINES

The quinine bi-salicylo salicylate was administered in the maximum tolerated dose (1.00 gm per kilo). The other substances were given in amounts equivalent to the quinine content of quinine bi-salicylo salicylate and, with the exception of the case of quinine salicylate, the salicyl content also equaled the amount present in this drug.

The drugs were introduced into isolated parts of the gastrointestinal tract, and in each case two hours later the blood was withdrawn and analyzed. The figures given in Table IX are the averages of several experiments.

TABLE IX

DRUG ADMINISTERED	QUININE FRACTION		SALICYL FRACTION	
	MG	PER 100 C C OF BLOOD	MG	PER 100 C C OF BLOOD
Quinine bi salicylo salicylate	Gastric absorption	2.6	9.0	
	Intestinal absorption	2.6	30.0	
Quinine salicylate	Gastric absorption	2.6	4.9	
	Intestinal absorption	3.8	6.3	
Quinine salicylate + enough sodium salicylate to make the salicyl equal to that of quinine bi salicylo salicylate	Gastric absorption	2.7	22.0	
	Intestinal absorption	3.2	49.1	
Quinine sulphate + sodium salicylate	Gastric absorption	3.0	28.0	
	Intestinal absorption	3.3	35.3	
Quinine sulphate + salysal	Gastric absorption	2.3	10.3	
	Intestinal absorption	2.6	25.4	

One of the remarkable facts that the above figures exhibit is the constant coefficient of absorption of quinine regardless of the form in which it is administered. The absorption of the salicyl fraction is more variable. Another interesting point is the low coefficient of absorption of salicyl from quinine salicylate as compared with quinine bi-salicylo-salicylate. When sodium salicylate, however, was added to the quinine salicylate in sufficient amount to bring the salicyl content up to the quantity present in quinine bi-salicylo-salicylate the salicyl absorption was greater than in any other case.

CLINICAL OBSERVATIONS

It is, of course, to the clinical and bedside impressions that we owe our estimate of the value of any drug, hence, our clinical observations in regard to quinine bi-salicylo salicylate, although limited in number, are not without interest.

Owing to the well-known fact that it is impracticable to make a detailed study of many upper respiratory infections, the only cases which were taken

for observation were those in which the temperature and other symptoms warranted continuing the patients to bed.

The number of cases observed was 80. This series included 10 cases of acute tonsillitis, 20 of acute pharyngitis, and 20 cases of influenza.

The patients with acute tonsillitis exhibited in every case rather severe symptoms, namely, a temperature of 103° to 104° F., marked dysphagia, general body pains, and malaise. They all showed the usual signs of severe tonsillar infection, and in every case the bacteriologic report of the throat cultures was positive for streptococci. The initial blood counts of WBC were from 12,000 to 18,000.

After 12 doses of 12 grams of quinine bisalicylo salicylate administered every two hours, marked relief was observed in the local and general symptoms, the temperature being normal in most cases. Thirty-six hours after the treatment was started the temperature of all the patients had returned to normal.

The 20 patients grouped under *acute pharyngitis* were also cases of the severer type. Their temperature ranged from 102° to 103.8° F. They all exhibited marked local reaction to the infective agent, and their initial blood counts of WBC ranged from 9,000 to 11,000.

The twenty cases of influenza were of the respiratory or febrile type. The sputum contained streptococci or pneumococci and occasionally *B. influenzae*. The WBC count was low. In thirty-six to forty-eight hours after the usual administration of quinine bisalicylo salicylate the temperatures were normal and the other symptoms were greatly ameliorated. All the signs of bronchial infection had cleared up in forty-two to seventy-two hours.

In none of the cases cited above was there any toxic reaction associable to quinine or salicylate.

On comparing this series with cases treated with other salicylates it was noticed that quinine bisalicylo salicylate acted more quickly in relieving symptoms and reducing temperature, and there was no accompanying gastric distress.

SUMMARY

From the various data that have been obtained from the foregoing experiments with quinine bisalicylo salicylate and related substances the following outstanding facts have been observed:

1. Quinine bisalicylo salicylate is very insoluble in neutral aqueous solutions.
2. In aqueous solutions of varying P_H it was found that quinine bisalicylo salicylate is most soluble in alkaline solutions of about P_H 10, next in acid solutions of about P_H 1, and least soluble in neutral solutions of P_H 7.
3. The dissociation fractions of quinine bisalicylo salicylate exhibit the chemical identification tests of quinine and salicyl.
4. The diffusion of quinine bisalicylo salicylate is greatest at P_H 10 and next at P_H 2.
5. Albumin does not interfere with the hydrolysis and diffusion of quinine bisalicylo salicylate.

6 The dialysis of quinine sulphate plus sodium salicylate from solutions of different P_H presents quite a different picture from the results obtained with quinine bi-salicylo salicylate. In this case P_H 7 is the reaction point at which the salicyl fraction is most diffusible and the maximum diffusion takes place within thirty minutes. The quinine fraction is diffusible in acid medium only, and the diffusion starts after two hours.

7 Albumin has a greater retarding effect on the diffusion of quinine sulphate and sodium salicylate mixture than it does on quinine bi-salicylo-salicylate.

8 The experiments on the elimination of the cleavage products of quinine bi-salicylo salicylate from normal human beings show that the salicyl fraction can be detected in the urine in about thirty minutes and continues to be eliminated for about sixty hours. The quinine fraction is first detected in about ninety minutes and ceases to be detectable after eight hours. The maximum elimination point of the two fractions is at about four hours.

9 The cleavage products of quinine bi-salicylo-salicylate (quinine and salysal) can be determined quantitatively. A method for their estimation has been devised.

10 The maximal tolerated dose and minimum lethal dose of quinine bi-salicylo-salicylate are greater than those of quinine sulphate plus sodium salicylate, quinine sulphate plus salysal, quinine salicylate plus sodium salicylate. In other words, quinine bi-salicylo salicylate is less toxic.

11 Quinine bi-salicylo salicylate and other quinine and salicyl mixtures caused a drop in body temperature when administered to animals, but no proportionality between the amount of drop and the dosage could be observed.

12 The respiration is very slightly affected with nontoxic doses of quinine bi-salicylo salicylate and related products. With toxic doses the respiration exhibits a marked increase. When lethal doses are given the respiration increases appreciably for several hours before death, but just before death it decreases.

13 The coefficient of adsorption of quinine is remarkably constant when administered to rabbits in the form of quinine bi-salicylo-salicylate, quinine salicylate, quinine salicylate plus sodium salicylate, quinine sulphate plus sodium salicylate, and quinine sulphate plus salysal. The absorption of the salicyl fraction of these substances is much more variable.

14 All the distinctive characteristics of quinine bi-salicylo-salicylate combine to make it an unusually valuable drug, but, perhaps, the most important are its nonirritating effects in the stomach in moderately large doses, its relatively low toxicity and its high salicyl content.

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STUDIES IN THE ALIMENTARY CANAL OF MAN*

VIII THE LIVE RELATIONSHIP OF GASTRIC PERISTALSIS

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INTRODUCTION

IN OUR studies upon the activity of the human stomach as observed by roentgenoscopic methods we have repeatedly observed the initial phenomenon of the appearance of peristalsis and have described it in the following terms. We make quite naturally the reservation that these phenomena do not invariably appear in complete expression and may indeed sometimes pass through successive phases so quickly as to be unrecognizable or apparent only as a foreshortened sequence with phases omitted.

When a five ounce fluid meal at 70° F. is administered to a resting stomach the immediate consequence is transmission of contents right through the pylorus and the neutral waves (hunger contractions) are delineated. This immediate passage ceases however before the end of one minute. The exact duration of the passage and also of the neutral waves depends upon the type of meal swallowed. There is then a pause when neither movement of stomach nor passage of contents can be seen. After the pause indentations occur in the gastric wall. They are sometimes visible on both curvatures depending upon the completeness of barium delineation but always appear along the greater curvature. They vary in number but occur typically at junction of pyloric canal with pyloric vestibule, at junction of pyloric vestibule with gastric tube and midway along the gastric tube. After an appreciable pause these indentations begin to pulsate and then after a varying interval, send off waves of peristalsis in the aboral direction. The first wave starts typically at the distal pulsation two minutes after the administration of the meal, the second at the intermediate and the third at the proximal pulsation. If conditions are favorable in that the upper gastric tube is sufficiently delineated, the fourth wave is seen to start high up in the gastric tube.

After the appearance of waves, one or more, it may be all of the pulsations disappear though their sites generally remain recognizable by a slight contraction of the greater curvature.

At any time any wave may disappear at a pulsation site and reappear after a few seconds, at the next. This phenomenon is sometimes interpreted as an observation that a wave may start up at any pulsation site not necessarily the highest. It is however perfectly true that in certain studies we have noted interpolated waves originating at divers sites along the lower gastric tube and the pyloric vestibule.

The regular rhythmic succession of waves may break down into apparently purposeless wavelets which we have defined as shimmer. There is no

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clear distinction between shallow indefinite peristalsis and shimmer. The latter simply represents the former without apparent force or directive purpose. It is most frequently seen after exhibition of a meal of low stimulative power like milk but there is also reason to believe that shimmer is sometimes induced by a subconscious nervousness perhaps unrecognizable by the patient. It is apt to follow a sudden touch on the abdominal wall by the observer's hand. It frequently replaces peristalsis early in the examination or when the patient is brought back to the screen after an interruption in the examination. It also is frequently seen to last for about half an hour after the administration of the meal in the first fluoroscopic examination undergone by the patient.

Temporary absence of peristalsis is itself probably often a subconscious nervous phenomenon for even in a practiced patient there may be no waves for a period of not more than two minutes from the commencement of roentgenoscopic examination when he is brought back to the screen after being disturbed for the making of a roentgenogram.

Compared with the amplitude of waves gastric peristaltic rhythm is rather constant in its time relations. In making serial roentgenographic studies for analysis by cinematographic technique, our routine, based upon this conclusion, calls for one picture every ten seconds. So regular in time relationship is the rhythm of peristalsis after an experimental meal that the ten-second interval in roentgenography records such differences in rhythm as may be found after different types of meal.

To make a proper analysis of peristaltic time relations requires the devotion of a roentgenoscopic session to this theme alone. Since the same stomach *must be used in studying the influence of different meals the investigation must be spread over a considerable interval* so that time may be given to the patient to recover from any possible effects of prolonged roentgenoscopy. Further the interval must be one during which the student can be reasonably assumed to enjoy steady good health and, in the evenness of a routine life, to be relatively free from fatigue and worry. Clearly such conditions can be fulfilled only in the summer vacation.

From such occasional observations as could be made in the course of roentgenoscopic examinations designed for other purposes we estimated the time taken in passage of a wave along the stomach to the pylorus as approximately the following: from junction of pyloric vestibule and canal, 7 secs., from mid-vestibule, 16 secs., from junction of gastric tube and pyloric vestibule, 26-27 secs., from mid-gastric tube, 35 secs., from Magenblase (fundus) 60-62 secs. It was intended to replace these by more accurate determinations when opportunity should arise. Having had this chance to secure the necessary data we are now able to present the facts which are indeed essential for the adequate further prosecution of our gastric investigations.

TECHNIQUE

The method followed has been to administer a large meal in order to define the stomach outline as high up the gastric tube as possible and also to maintain the clearness of definition as long as possible. It has been found that, after some meals at least, buttermilk for example, there is progressive in-

crease in peristaltic amplitude during the first ten minutes. We have also shown to our satisfaction that entry of the stomach into the rather quiescent neutral phase characteristically commences twenty minutes after a five ounce meal is much delayed by exhibition of a large meal. We have therefore administered large meals consisting of sixteen ounces of vehicle and four ounces of barium sulphate. Roentgenoscopic observations began in each study fifteen to thirty minutes after the meal was swallowed. One of us (WAS) acted as the subject of these experiments and was entirely responsible for working up the data; another (WMK) made the time determinations and checked the records set down by the third (TWI) who is responsible for the final form of this manuscript. The initial observations upon which this study was planned were carried out by WMK and checked by TWI. The subject is quite experienced in roentgenoscopic examinations and has had many investigations made upon his own stomach including two serial studies. His age when the study commenced was 21 years, his height 1747 mm, and his weight 207 pounds. The conditions of health and routine mentioned above have been properly fulfilled. The observer (WMK) sits in front of the roentgenoscopic screen and notes the first appearance of a wave of which the progress is to be followed. She calls out the site where the wave is first noted and the exact time is registered by the recorder (IWP) who has before him a stop clock with a second hand. As the observer traces the progress of the wave she calls out the arrival of the wave in each successive segment or level of the stomach. The times are duly recorded to the nearest second and thus the time relationship of the wave is registered. This method was adopted after first attempting to make the record directly by means of a dictaphone and with the aid of a ridium faced stop clock. The maneuvers necessary to watch roentgenoscopic screen and stop clock at the same time proved too complicated and the time was better recorded in the manner described as finally adopted.

In order to avoid injury to the subject, each roentgenoscopic session has been limited to the registration of approximately ten waves and some time has been allowed for the subject to recover before the next session was scheduled.

In this study we decided to compare the time relationship of peristalsis after six large fluid meals. These are water, milk, buttermilk, lactic acid solution in water buffered with sodium hydroxide to pH 4, sp. menth. pip. 20 minims in sixteen ounces of water, and sodium bicarb. 60 grains (4 grams) in sixteen ounces of water. Thus each large meal consisted of sixteen ounces of fluid together with four ounces of barium sulphate. Each meal is given at a temperature of 70° F. since it has been shown that raising or lowering the temperature of the gastric contents modifies the peristalsis.³ The average time relationships have been calculated and the standard deviations computed from the formula derived by "Student" for a very small series.²

The effects of application of heat and of cold to the abdominal wall upon gastric behavior pattern were also included in our study and are recorded. A hot pack or an ice bag was held to the abdominal wall for a period of forty-five minutes. A twenty ounce milk-barium meal was administered thirty minutes after the pack or bag was first applied to the abdominal wall and

fifteen minutes before the examination. Then, the subject standing behind the roentgenoscopic screen, the pack or bag was removed, and the gastric behavior pattern watched at frequent intervals for half-an-hour.

The eight studies here recorded were spread over two successive summer vacations in order that there might be no possible detrimental effect from prolonged and often-repeated x-ray exposures and also that the mental and physical health requirements laid down above might be maintained.

In our subsequently recorded observations the gastric subdivisions used are the junctions of gastric tube with pyloric vestibule and of pyloric vestibule with pyloric canal. The gastric tube and pyloric vestibule are each subdivided into upper, middle and lower sections and the time registered when the wave passes over the center of each.

RECORDS OF GASTRIC BEHAVIOR, CHEMICAL STIMULI

The qualitative observations made at the successive sessions are important for establishing the entirely regular behavior of the stomach under examination. The dimensions of the gastric shadow depend not so much upon the amount of the experimental meal as upon the interplay of pyloric action and gastric secretion called forth. Water, for example, results in an immediate profuse gastric secretion but, owing to continued patency of the pylorus which, after water, in our experience, never completely closes, the stomach shadow remains relatively small. The water-stomach shows a motor response which is relatively more evanescent than those consequent upon other fluid stimuli. According to our observations a five-ounce water meal is flushed from the stomach by gastric juice in about ten minutes whereas all the other fluids, studies of which are here recorded, betray their presence by a characteristic response for about twenty minutes after administration. We started the observations upon effect of water stimulation about twenty-five minutes after administration of the large meal and continued them until fifty minutes after administration in order to note the effect of the flushing process upon the wave time as the water became more diluted and finally displaced by the gastric secretion. In this we were disappointed since the method of recording does not permit such fine distinction but the standard deviation in Table I gives a rough measure of change of wave time.

Water meal. The entire stomach outline was clearly seen and the gastric tube very narrow, consistent with the patent pylorus above mentioned. Several waves were present at one time, giving an impression of unusual speed. The waves were of considerable amplitude, obvious on both curvatures and almost pinched the barium shadow in two.

In Table I may be seen the average length of time which the wave takes to reach the pylorus from each gastric level beyond the fundus, together with the variability in this time. It is important to observe, first, that, after water, waves can be traced usually from mid-gastric tube and occasionally from upper gastric tube. Secondly, the speed of waves in a water stomach is greater than after any one of the other five fluid stimuli with the exception, in the pyloric part, of peppermint. The speed of water-stimulated peristalsis is therefore a standard against which to check the peristalsis induced by other forms of stimulation.

TABLE I
TIME IN SECONDS TAKEN BY WAVE TO TRAVEL TO PYLORIC AND ILEOCECAL JUNCTIONS
WITH STANDARD DEVIATIONS

TRAVEL	PERITUMENT			WATER			MILK			WITHEUMIN			SODA			TOILET		
	NO WAVE TIMED	MEAN	S.D.	NO WAVE TIMED	MEAN	S.D.	NO WAVE TIMED	MEAN	S.D.	NO WAVE TIMED	MEAN	S.D.	NO WAVE TIMED	MEAN	S.D.	NO WAVE TIMED	MEAN	S.D.
Upper G.F.	1	44.0	—	1	39.0	—	—	—	—	2	39.0	—	1	41.0	—	—	—	—
Middle G.F.	2	35.4±1.34	2.50	7	32.0±0.72	2.14	—	—	—	1	30.0	—	6	31.1±1.70	2.25	—	—	—
Lower G.F.	1	29.0	—	7	27.1±0.85	2.15	2	29.0	—	8	23.1±0.84	2.13	5	25.0	—	—	—	—
In G.F. & PY	10	35.8±0.70	1.34	11	23.4±0.61	2.09	7	24.5±0.40	2.06	11	29.0±0.61	2.17	10	27.5±0.66	1.75	10	21.6	1.24
Upper PY	9	17.7±0.91	1.46	11	17.5±0.31	2.01	9	18.0±0.34	1.62	10	20.1±1.31	1.46	9	21.6	0.71	11	21.1	0.81
Middle PY	10	11.9±0.58	2.74	13	12.2±0.15	2.12	11	13.0±0.30	1.41	10	13.1±0.21	1.81	10	15.1±0.15	0.11	11	15.1	0.82
Lower PY	10	7.3±0.16	1.50	13	8.0±0.19	2.61	11	8.1±0.24	0.90	9	8.5±0.11	1.57	10	10.0±0.11	1.15	11	12.2	0.71
In PY & PC	10	3.7±0.11	1.85	12	1.5±0.20	1.04	11	1.1±0.16	0.77	10	3.2±0.15	1.61	10	1.0±0.00	1.25	8	0.5	0.48

C.F. Gastric tube PY Pyloric vestibule PC Pyloric canal In Junction

TABLE II

AVERAGE TIME IN SECONDS FOR PASSAGE OF WAVE FROM ONE GASTRIC LEVEL TO THE NEXT

	PEPPERMINT	WATER	MILK	BUTTERMILK	SODA	LACTIC ACID
Upper GT	8.0	5.0		10.5	3.0	
Middle GT	7.0	4.9		8.0	7.0	
Lower GT	5.0	4.0	7.0	4.0	5.5	6.0
Jn GT and PV	7.9	6.0	6.5	5.6	7.0	7.2
Upper PV	6.6	5.4	5.3	7.5	6.0	7.0
Middle PV	4.6	4.2	4.7	3.8	5.1	5.3
Lower PV	3.6	3.8	3.9	3.0	4.6	5.5
Jn PV and PC	3.7	4.5	4.4	5.2	5.4	5.8
Pylorus						

Number of observations and verbal contractions as in Table I

Table II gives the average time in seconds occupied by a wave passing from one gastric level to the next. This table has been built up from averages of the actual records and not by subtraction of one average of Table I from another. Roughly the intervals are five seconds except in distal pyloric vestibule and pyloric canal when, the distances being shorter, the intervals are less. The water-stimulated wave passes regularly along the stomach without pauses such as are observed in peristalsis stimulated by other contents. The water-waves do not weaken as they proceed, do not fade out temporarily or break down into shimmer.

Milk Meal—When the stomach was first observed, twenty minutes after the meal, the barium had settled and the gastric outline was clearly apparent from pylorus to upper pyloric vestibule. The Magenblase was flat and the gastric tube shadow relatively wide in comparison with its diameter after the water meal. This difference in gastric tube width is due to secretion of gastric juice stimulated by the milk but held in the stomach as a result of the pyloric rhythm which brings about alternate opening and closure of the pylorus in place of the practically continuous patency after water administration. Milk waves are shallow and very apt to break down into shimmer. A faint shadow of intermittent passage through the pylorus was noted.

Reference to Table I demonstrates that there is no difference in speed between milk-stimulated waves and water-stimulated waves except in the lower gastric tube. Statistically one would not say that there is a difference even here. But experience assures us that speed is less rapid about the junction of gastric tube and pyloric vestibule than in the pyloric area when the peristaltic waves are milk-stimulated. Indeed we had difficulty in observation at the milk session, through waves fading out about this level or breaking down into shimmer or even being obscured and inhibited in a pulsating ring at the junction of tube and vestibule.

From Table II one learns that the loss of time resulting from this reduced speed averages about three seconds. Our unpublished records also show a faint suggestion of the retardation carrying over into upper pyloric vestibule.

Buttermilk Meal—When the observations were first made twenty-five minutes after the meal the entire stomach outline was clearly visible. The gastric tube was very wide as is usual after buttermilk owing to the rapid outpouring of gastric juice. Further this great breadth of gastric tube is not due to a simple anteroposterior compression of the stomach for we turned the patient side ways and observed that the gastric tube shadow was the same breadth from before backwards as from side to side. The stomach was adjusted to approximately the same distance from the screen in both views. The usual dense shadow of piling was noted after every wave.

Table I shows a marked reduction in speed of waves through the gastric tube compared with the speed of water and milk stimulated waves but no such slowing up in the pyloric part. It is apparent that the slight pause or slackening of speed noted about the junction of tube and vestibule in the milk stomach was but the tail end of the reduction of speed in gastric tube which we have now had the opportunity to observe after buttermilk. The amplitude of milk stimulated waves is very small but that of the waves after water or buttermilk is quite marked. Hence speed and amplitude have no relation to each other. The difference between milk waves and buttermilk waves is more clearly seen in amplitude; the difference between buttermilk waves and water waves is rather one of speed. It is not to be assumed however that we intend to make any special distinction in pattern of rhythm between gastric tube and pyloric vestibule. Slowing of the rhythm is best marked and earliest noted at the fundic end of the stomach and spreads aborally. After a milk meal the slowing is confined entirely to gastric tube but as Tables I and II both show after a buttermilk meal the speed tends to slow down in vestibule also.

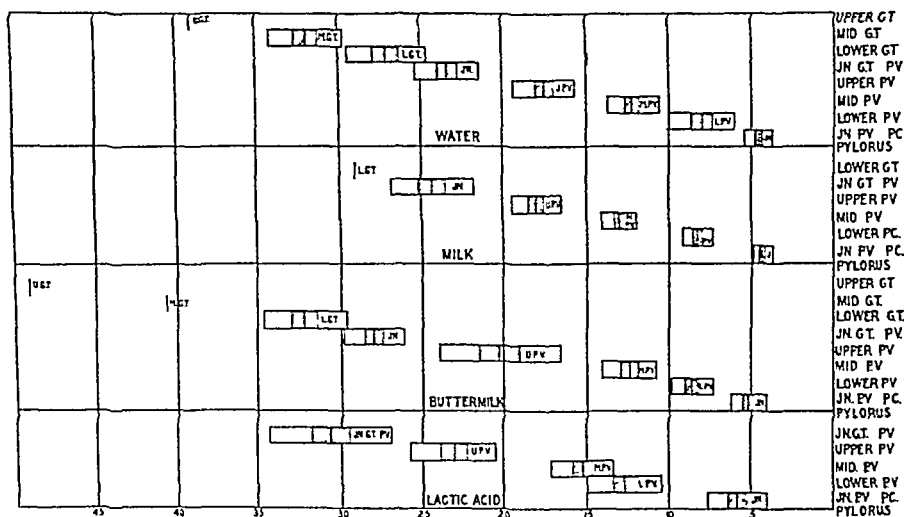
Lactic Acid Meal—Quarter of an hour after taking the meal the gastric tube was moderately broad from secretion of gastric juice and waves could be observed deep and numerous along the greater curvature. Unfortunately, gas in the splenic flexure prevented us from making observations proximal to the junction between gastric tube and pyloric vestibule. However this handicap is not so severe as it might seem because the record indicates a slowing of rhythm all the way along the stomach so far as our observations were possible. Both Tables I and II indicate this general slackening of speed which, upon the basis of the buttermilk pattern, may be inferred also in the gastric tube where conditions of the experiment denied us direct observations.

Reference to Table I emphasizes the erratic time-relationship of lactic acid waves. The standard deviation is usually about twice that of the buttermilk waves at identical gastric levels. This is but one example of the character of the lactic acid behavior pattern. We have been greatly puzzled to interpret our observations on lactic acid. In some stomachs the behavior simulates that of buttermilk, the gastric shadow especially in the tube, is large, the waves are slow and their amplitude great. In other stomachs the lactic pattern is more nearly that of water, the gastric shadow is narrow with both ballooning and cog wheel effects so typical of water, the waves are short and rapid and their amplitude moderate.

Soda Meal—It was not until the following summer (1930) that the remaining experiments here recorded were undertaken. With so long an inter-

val as a year elapsing between the two groups of experiments it was necessary to insure that the general condition of the stomach had not changed in the interval. We therefore used our "outliner" to give us an observation of gastric activity before starting the experiment. This outliner consists of five grams of barium sulphate in half an ounce of water. It is just enough to outline the greater curvature and delineate the waves without affecting them for more than five minutes, since this small quantity of water is very rapidly flushed through the pylorus by secretion of gastric juice.

Having ascertained that the condition of the stomach was unchanged from the previous summer, we administered a large soda meal, the composition of which has been stated. In this subject the entire gastric tube and pyloric vestibule were distended, as frequently, but not invariably, occurs after the exhibition of this drug. Gas in the splenic flexure interfered with



Figs 1 and 2.—Graphic presentation of the phenomena recorded in Tables I and II.

The distances of the central thick lines from the right hand ordinate represent the average time taken by a wave to travel to the pylorus from that particular gastric level. Verbal contractions as in Table I.

The hatched areas indicate the probable error on each side of the mean and the unshaded rectangle delimits the value of the standard deviation. The progressive slackening of speed in the milk, buttermilk, lactic acid, and soda series is very clearly shown.

observations upon the waves in the upper gastric tube. The waves however were quite definite, deep, forceful, slow soda waves.

Reference to Table I shows that the progress of the soda wave is relatively slow throughout its entire course. The speed of passage over the stomach wall is very similar to that of buttermilk and the variability in time of successive waves is again very similar to that of buttermilk. In form, however, the waves could not be mistaken for buttermilk waves for their outline is undulating and forceful rather than constricting and vigorous.

Peppermint Meal—On the day after the soda pattern was studied, a large peppermint meal, of the composition noted above, was given after a regular outliner had been administered to identify the gastric response. The Magendie, gastric tube and pyloric vestibule remained rather narrow as our studies have led us to expect after peppermint administration. The resulting

the effect of both heat and cold is an increased gastric activity as shown by amplitude of waves and rapidity of peristalsis. The effect of cold is much shorter in duration than that of heat. Whereas the depth, speed, frequency and vigor of peristalsis induced by heat may continue in diminished degree for fifteen or even twenty minutes, the stimulant effect of cold has already faded at the end of five minutes.

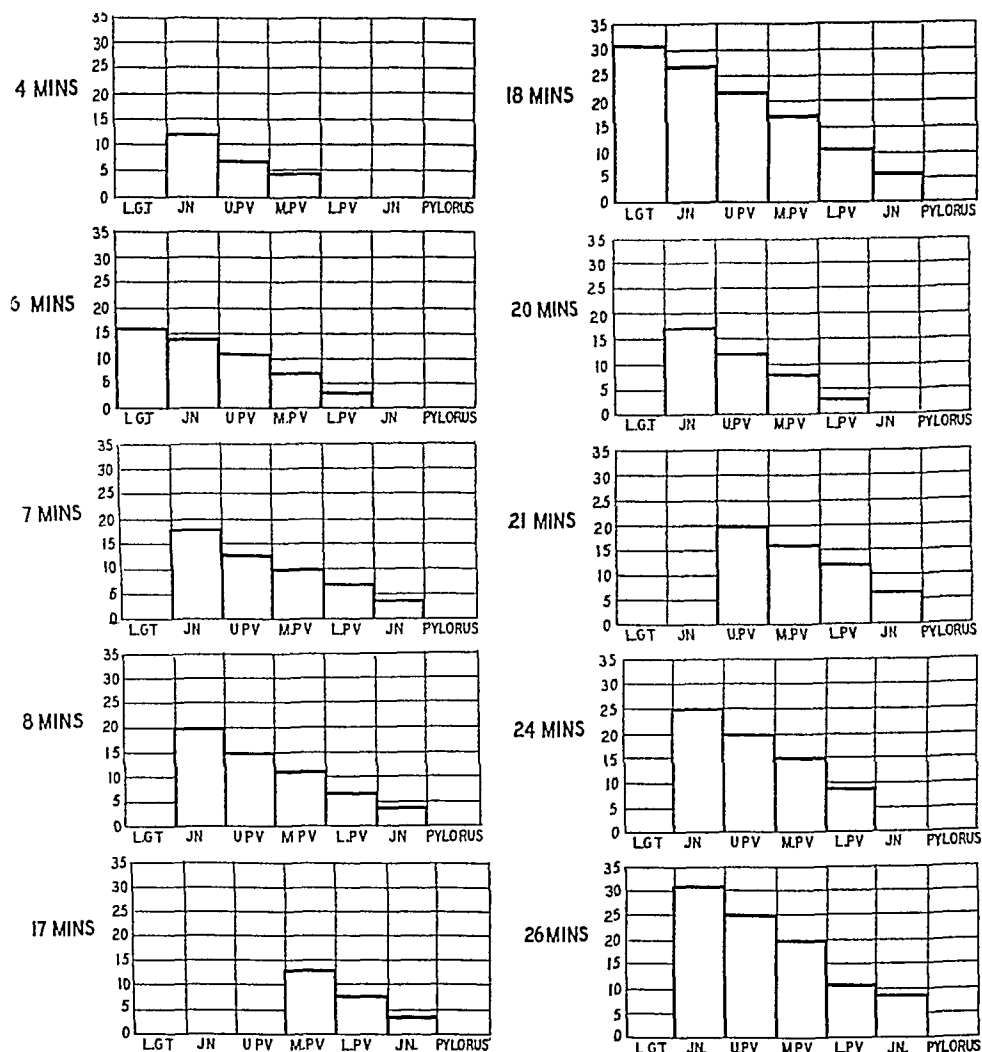


FIG 3-A

FIG 3-B

Fig 3—Serial record of gastric waves after the application of external heat. Four minutes after cessation of heat application to abdominal wall the speed is still three times that of normal water-stimulated waves. The speed progressively diminishes until about eighteen minutes after removal of stimulus. Abscissae in seconds. Verbal contractions as in Table I.

The technique of observation was essentially different from that in the foregoing studies. We were not now concerned in the variability of a rather constant type of rhythm which indeed developed to a maximum at some minutes after the observations began. We now had to face a rhythm, the initial

expression of which would be most typical and vigorous and the later contraction less typical in appearance. Although we could not prolong the experimental session we made observations at approximately every two minutes after the withdrawal of the hot pack or the ice bag from the abdominal wall and have recorded these in Figs. 3 and 4. In order to facilitate comparison

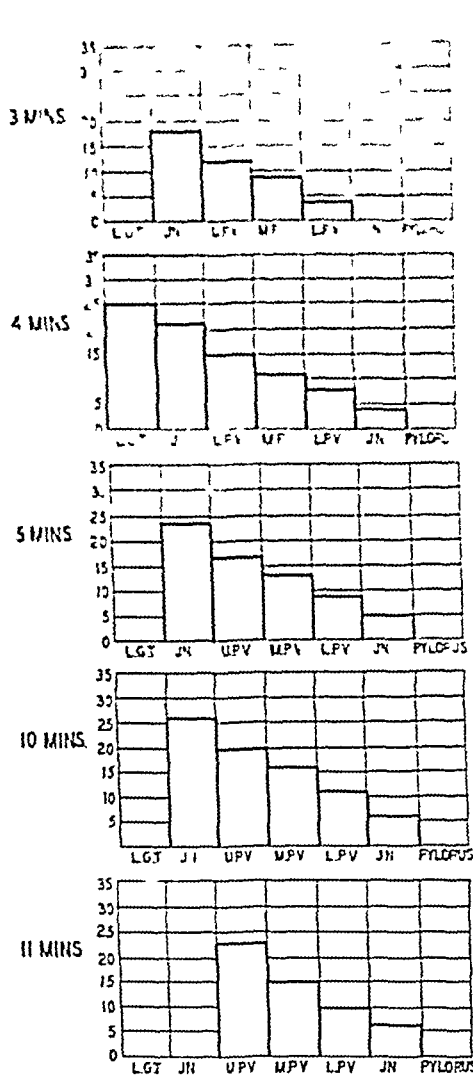


Fig. 4-1

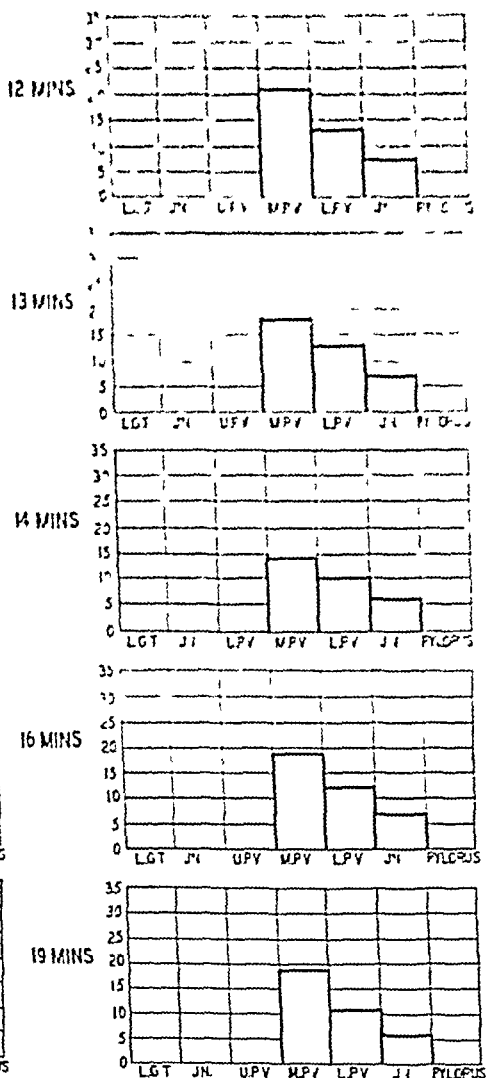


Fig. 4-B

Fig. 4—Serial record of gastric waves after application of external cold

Five minutes after cessation of cold application to abdominal wall the speed originally induced by cold is reduced to that of ordinary milk waves. A further reduction in speed occurs until the expiration of ten minutes. Abscissae in seconds. Vertical contractions as in Table I.

with the effect of chemical stimulation we have introduced Table III which gives the total average length of time taken by the wave induced by the different chemical stimuli to reach the pylorus from the successive gastric levels. Table III is directly comparable with Figs. 3 and 4.

TABLE III
COMPARISON OF WAVE TIMES TO PYLORUS IN SECONDS

	UGT	MGT	LGT	JN	UPV	MPV	LPV	JN
Peppermint	44 0	35 5	29 0	26 0	17 5	12 0	7 5	3 5
Water	39 0	32 0	27 0	23 5	17 5	12 0	8 0	4 5
Milk			29 0	24 5	18 0	13 0	8 5	4 5
Buttermilk			32 5	28 0	20 5	12 5	8 5	5 0
Soda	41 0	38 0	32 0	27 5	21 5	15 0	10 0	5 5
Lactic Acid				30 5	23 0	15 5	12 5	6 0

Owing to the difficulties in observation it was not always possible to examine the wave throughout the course of the stomach. Our records in Figs 3 and 4 are therefore somewhat patchy, they do however give a quite clear impression of the fading effect of heat or cold upon the gastric mechanism.

The reader may wonder why we applied heat and cold to the abdominal wall instead of making use of hot or cold drinks. In our previous article³ we have pointed out that the indirect or reflex effect of heat and cold through the abdominal wall is more vigorous and typical than that obtained by the exhibition of hot and cold drinks.

Effect of Heat—For several minutes after the observations were begun we were unable to time the gastric waves, the passage of which, at least in the lower part of the stomach, was very rapid. Four minutes after the observations began the passage of a wave from junction of gastric tube and pyloric vestibule to pylorus took only twelve seconds as against twenty-four seconds for water or milk. The speed of the heat wave along the pyloric vestibule was so great that four seconds only were occupied in passing from mid-vestibule to pylorus as against the twelve seconds characteristic of water, milk, peppermint and buttermilk. Thereafter, the speed of the heat waves diminished until about eighteen minutes after the observations began, at which time, as indicated in Fig 3, the speed was practically that of milk at 70° F. It is true that occasional irregularities appear as illustrated in the wave recorded twenty minutes after commencement of the study.

We continued this examination for twenty-six minutes and found that, during the last ten minutes of the examination, the gastric waves became unusually slow. It is not fair to state that this was due to the superintention of the neutral pattern. In the first place the neutral pattern does not assert itself so rapidly in a stomach containing a large meal. In the second place the time relationship of neutral waves is practically that of the water pattern. One must therefore conclude that, after eighteen minutes, the vigor of gastric peristalsis induced by heat gives place to a rather lethargic rhythm.

Effect of Cold—The examination of the gastric pattern reflexly stimulated by application of cold to the abdominal wall follows exactly the lines of that adopted for heat. We were faced with the same difficulty at first, namely, that the speed of the early waves was so great that they defied exact time measurement. At the end of three minutes however, the time taken by a wave to pass from the junction of gastric tube and pyloric vestibule to pylorus was eighteen seconds as against the twenty-four seconds taken by milk or water waves to travel the same distance. From the mid-pyloric vestibule the wave occupied eight seconds instead of twelve or thirteen. This activation by cold

is then much less vigorous than that induced by heat. An inspection of Fig. 4 demonstrates the rather rapid diminution in speed of the waves induced by cold so that at the end of five minutes from the commencement of observations the time relationship is practically that of milk at 70° F. Thereafter until some eleven minutes after removal of the cold pack there was a progressive slowing in the wave time. During the remainder of the study the time relationship of gastric waves remained unchanged.

CONCLUSIONS

Our series of eight sessions has helped us considerably to understand the phenomena of gastric behavior. It is true that we have had only one stomach under special observation. But this was a carefully trained and stabilized organ whose general behavior pattern we know from repeated study under different conditions. Moreover the results of our investigation of this stomach confirm and extend the occasional quantitative observations which alone have been possible to us in the past.

The water stimulated stomach should be taken as the standard because its behavior is practically that of the neutral organ, because wave amplitude is great and waves can be followed from upper gastric tube with ease, and also because the wave travels with a steady rhythm throughout its course and does not tend to fade out or break up into a shimmer.

The milk, buttermilk and lactic acid groups of observations form a definite series, but in amplitude milk waves are very shallow and without the vigorous character of buttermilk waves or the erratic vigor of the lactic acid waves. The series is one of rhythm progressively slackening. Milk stimulated waves are slightly slower in their speed of passage over gastric tube but progress in pyloric vestibule is exactly similar to that of water stimulated waves. After buttermilk this slackening of speed is continued into the vestibule. After lactic acid it becomes general and marked throughout the stomach.

The soda waves, apart from their deep and forceful character are similar to those of buttermilk in their time relationship. These peppermint waves on the contrary, have approximately the neutral character in common with those of water and of milk throughout the pyloric part of the stomach. They are however definitely slow in the gastric tube and seem to pause more distinctly than those of soda and of lactic acid at the junction of gastric tube and pyloric vestibule.

The waves induced by heat are at first vigorous and very rapid. The effect is not lost until the lapse of about eighteen minutes after removal of the hot pack. Thereafter the time relationship is lengthened as though the waves become lethargic in character. Waves stimulated by cold diminish in speed so rapidly that five minutes after removal of the cold pack their distinctive character is lost. They continue to diminish in speed until the end of about ten minutes when they become stabilized.

Further study is needed to elucidate the cause of these differences in gastric behavior pattern and further observations are essential in order to establish their actuality. We are recording these results now because we feel convinced, in the light of our experience, that the phenomena herein de-

scribed are definite and will be confirmed by careful observers upon trained and stabilized stomachs

SUMMARY

The rhythm of gastric peristalsis is definitely influenced by the particular stimulus which initiates it. Water produces a regular active peristalsis.

Milk-stimulated waves differ from those produced by buttermilk and lactic acid in being of low amplitude, but in rhythm they fall into series with the waves of buttermilk- and lactic acid-stimulated peristalsis.

Milk-stimulated peristalsis is slightly slowed in speed in the gastric tube but not in pyloric vestibule. The speed of buttermilk-stimulated waves is slightly slackened in pyloric part as well as in gastric tube. But the lactic acid meal produces waves very definitely reduced in speed throughout their progress from upper gastric tube to pylorus.

Soda waves are deep and forceful and their time relationship is very similar to that of buttermilk-stimulated waves.

These peppermint waves, like water and milk waves, are almost indistinguishable in time relationship from the neutral pattern in the pyloric part of the stomach. They are easily distinguished from the neutral pattern by their greater amplitude and vigor. In the gastric tube they are distinctly slow like the soda waves.

The waves stimulated by soda, peppermint and lactic acid appear to pause at the junction between gastric tube and pyloric vestibule.

The influence of heat and cold differs essentially from that of the chemical stimuli above recorded in that the initial effect is the most powerful and there is a constantly diminishing degree of expression whereas the effect of a chemical stimulus rises to a maximum a few minutes after its earliest expression.

The waves induced by heat are at first so rapid that the record of their time relationship is very difficult. At the end of four minutes the speed is still about three times that of water-stimulated waves. The speed progressively diminishes until about eighteen minutes after the heat stimulus has been removed. The stomach then apparently enters a lethargic phase.

The effect of cold is similar to that of heat but far less pronounced. By the end of five minutes the speed of waves induced by cold has become reduced to that characteristic of an ordinary milk meal at 70° F. Until the end of ten minutes there is a progressive slowing in the wave time but thereafter the time relationship is unchanged.

REFERENCES

Instead of referring to definite paragraphs of previous articles from this laboratory, a very difficult and involved procedure, we give a list of references to the more important contributions bearing upon the problem in hand. A general summary has been given of our observations to date by the senior author in his recent book on the Behavior Patterns of the Alimentary Tract published in 1930 by Williams and Wilkins, Baltimore.

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AN ANALYSIS OF FIFTY HEART CASES SHOWING LOW VOLTAGE*

BY RUSSELL N. STECKMAN,[†] M.D. AND MURRAY L. RICH,[‡] M.D.
CINCINNATI, OHIO

A REVIEW of the literature has shown conflicting reports as to the significance of electrocardiograms of low voltage—that is, with R waves of 5 mm. or less in all leads. Master and Purdee¹ stated that a voltage of the QRS group so low that its size does not exceed five mm. in the lead of largest excursion is a significant abnormal finding. Hopburn and Jamieson² in a series of 34 patients stated that there is no doubt as to the gravity of low voltage electrocardiograms as a prognostic sign, and that the only other group with a higher mortality is that of bundle branch block. Sprague and White³ in a study of 57 patients stated that they had never observed electrocardiograms of low voltage in patients with normal hearts. They concluded that it is a finding of diagnostic and prognostic importance in forming an opinion of the myocardial ability of any individual. Williams and Killins⁴ reviewed 140 patients presenting electrocardiograms of low voltage. All their electrocardiograms showed low voltage alone; records displaying other significant abnormalities were rejected. They stated that it is not justifiable to conclude that electrocardiograms of low voltage unassociated with other graphic abnormalities indicate serious myocardial disease or are of serious prognostic importance.

During the past three years approximately two thousand electrocardiograms have been taken on the Medical Service of the Cincinnati General Hospital. These tracings have not been taken routinely on all patients, but only on those in whom there was either definite or questionable evidence of heart disease. Of this number 50 cases showed low voltage, and our study comprises a review of these 50 cases in detail. It is to be noted that these patients on whom these electrocardiograms were taken were practically all from the laboring class and as is usual with patients of this class, many of them showed evidence of marked heart failure on admission to the hospital.

The criterion which we have used for the diagnosis of low voltage was that the total deflection of the QRS complex did not exceed 5 mm. in any of the three standard leads. All the records were taken on an amplifying instrument with the usual standardization, that is, one centimeter deflection equivalent to one millivolt current.

Of these 50 patients, 47 entered the hospital because of symptoms referable to the heart. Their ages varied between twenty and eighty with about an equal distribution in each decade. There were 36 males and 14 females. The majority of the patients were white. Shortness of breath and edema of

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Of these 50 patients, 47 entered the hospital because of symptoms referable to the heart. Their ages varied between twenty and eighty with about an equal distribution in each decade. There were 36 males and 14 females. The majority of the patients were white. Shortness of breath and edema of

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the extremities were the leading symptoms. The duration of the illness was less than one year in 33 of the patients.

Arteriosclerotic heart disease was the etiologic factor in 30 of these patients, 16 of them having an associated hypertension. Two others showed hypertension alone. Seven were rheumatic, and there was one each of the following: Infectious, syphilitic, combined syphilitic and arteriosclerotic. One patient had a carcinoma of the lung with metastases to the pericardium and myocardium. In four, tuberculosis was the etiologic factor, two showing pericarditis with effusion and the two others an adherent pericardium, as proved at autopsy. In two, the etiology was unknown. In only one case was there no clinical evidence of heart disease.

The heart was enlarged in 42 of the patients. In 43 there was no valvular disease. Mitral stenosis was present in three patients, aortic insufficiency in two and mitral insufficiency in two. Pericardial effusion was present in 5 patients. Adherent pericardium was found at autopsy in three cases and was diagnosed clinically in two others. There was heart failure of the congestive type in 47 cases and of the anginal type in one other. Only four patients showed pulsus alternans. There were no cases of mixedema in our series.

We have separated the electrocardiograms showing low voltage alone from those in which low voltage was associated with auricular fibrillation, auricular flutter, inverted T-waves in the significant leads, aberrant QRS complexes, including incomplete bundle-branch block, any degree of heart-block or ectopic tachycardias. There were 19 patients showing low voltage alone. Of this number 16 are dead, two are untraced and one is still living. Of the number dead, 10 died within one month after the first electrocardiogram, showing low voltage and all were dead within a period of nine and one-half months.

Auricular fibrillation was associated with low voltage in 14 patients. Eight are dead, four are untraced and two are still living. Five died within one month after low voltage was first discovered and the other three died within ten months.

The QRS complexes showed aberration in all leads in 10 cases, 4 of which are classified as incomplete bundle-branch block. Of these, 9 are dead and one is untraced. Six died within one month, the remaining 3 within ten and one-half months.

Heart-block, including delayed conduction, partial or complete block, was associated with low voltage in 9 cases. Six of these are dead, 4 within one month and 3 are still living.

Seven cases showed inverted T-waves in the significant leads. Three died within two and one-half months, 3 are still living and one is untraced.

Twenty-five of these patients had more than one electrocardiogram. In 10 of these, the low voltage persisted in all tracings. In 8 others the voltage later returned to normal. Tracings had been taken in 7 before low voltage appeared. In 19 of these cases we observed that there was a correlation between the patient's condition and the voltage of the electrocardiogram, in that the voltage was greater as the patient's condition improved. In 6 other cases there was no such relationship. In one case with pericardial effusion the

voltage returned to normal following the removal of 900 cc. of fluid from the pericardium.

There were 36 deaths in the 50 cases which comprised this series. Seven of the patients are known to be living and 7 are untraced. Two of those known to be living are still in the hospital. Of the 36 deaths, 32 occurred within six months from the time the first electrocardiogram showing low voltage was taken. Twenty-six of these patients died in the hospital and autopsy was performed on 14. Heart failure was the cause of death in 26.

In the 14 autopsies the heart was found to be enlarged in 10. Pericardial effusion was found in 4, adherent pericardium was present in 3. The coronary arteries showed some degree of sclerosis in 10 cases. Microscopic examination showed abnormalities in the heart muscle in every case, the most frequent finding being fibrosis of the myocardium. This was present in 9 cases. Arteriosclerosis was present in 8 cases. All the cases with pericardial effusion showed coronary arteriosclerosis, myocardial fibrosis, or both.

SUMMARY

1. Forty-seven of the 50 patients showing low voltage gave symptoms referable to the heart. The duration of the illness was less than one year in 33. Forty-nine had clinical evidence of heart disease.

2. Arteriosclerosis, with or without hypertension, was the leading etiologic factor in our series.

3. In 19 cases low voltage alone was present. The mortality in this group was 16 or 84 per cent within ten and one-half months.

4. Fourteen cases showed auricular fibrillation in addition to low voltage. The known mortality of this group was 8 or 57 per cent within ten months.

5. Ten patients showed aberrant QRS complexes, including intraventricular block. The known mortality of this group was 9 or 90 per cent in ten and one-half months.

6. Nine patients showed heart block. Six or 67 per cent were dead in seven months.

7. Seven patients showed inverted T waves in significant leads. Three or 43 per cent were dead within two and one-half months.

8. There were 36 deaths in the series of 50 cases, 32 of the patients died within six months from the time the first electrocardiogram showing low voltage was taken.

9. Autopsies were performed on 14. The coronary arteries showed some degree of sclerosis in 10 cases. Microscopic examination showed the myocardium to be abnormal in every case, fibrosis of the myocardium being the most frequent finding.

CONCLUSION

We believe from the study of the above 50 cases that the occurrence of a voltage of 5 mm. or less regardless of other electrocardiographic abnormalities in a patient with heart disease is of serious prognostic import.

*We are indebted to Dr. Pearl M. Zuck of the Department of Pathology of the University of Cincinnati and the Cincinnati General Hospital for the autopsy reports.

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LABORATORY METHODS

A CRITICAL STUDY OF THE POLYNUCLEAR COUNT AS ADVOCATED BY SCHILLING*

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THE increasing interest in the Schilling method of the differential counting of the leucocytes has led the author to a critical study of the method in tuberculous cases. The method advocated by Schilling¹ is a somewhat simplified Arneth method. Both authors have recognized and emphasized the fact that a considerable amount of useful information may be gained from differential leucocytic counts if one takes cognizance of the variations in nuclear architecture of the neutrophils. Both authors have stressed the fact that the more the neutrophils are representative of the immature stage of that cell the more serious is the infection.

The most serious drawback to the method of both of these authors is that it is rather difficult to identify accurately all of the neutrophils as they have classified them. A considerable difference in personal judgment enters into any detailed classification such as they advocate. Realizing the value of this method others have sought to further simplify it. The most significant contributions toward a simplified classification have been made by Pons and Krumbhaar, Pincus, Cooke and Ponder, and Farley, et al.²

Our study was concerned with the changes in the nuclear variations of the neutrophils from day to day to determine, if possible, the real significance of the younger cells in the circulation. We were interested to find out whether a marked increase of young forms in one count over those in a previous count would indicate a more serious condition of the patient. To simplify the classification down to a minimum we have placed all nonsegmented nuclear forms of the neutrophils into Class I and all segmented forms, regardless of the number of segments, into Class II. With this modification the only consideration is whether the nucleus is segmented or nonsegmented and the differences in personal judgment are reduced to a minimum. The following table will show in what way this classification has condensed that of the authors mentioned above. This simplified classification is the same as advocated by Farley, et al.

TABLE I

	CLASS I	CLASS II
Arneth	I	II, III, IV & V
Schilling	I, II & III	IV
Pons and Krumbhaar	I & II	III

*From the Hageman Research Laboratory of the Metropolitan Life Insurance Company Sanatorium.

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Our study included 100 individuals who were patients in the sanatorium. Repeated counts were done on each individual. The smallest number of counts made on any case was four. In the large majority of individuals ten to twenty counts were done. Our study extended over a period of six months and about 1,200 counts were made. All of the differential counts were made by the author in order to have as uniform results as possible and to eliminate the factor of judgment on the part of others.

The blood smears were made on cover-slips and were stained with Wright's blood stain. Four hundred cells were counted in every instance. The total leucocytic counts were done in the usual way from a one-to-twenty dilution of the blood in 0.1 per cent acetic acid solution.

The majority of the cases in our study were proved cases of tuberculosis. We had the opportunity, however, to include in our observation individuals who were ill from diseases other than tuberculosis.

We have selected from our entire group nineteen cases to illustrate the leucocytic pictures which we have encountered in various diseases and in different stages of tuberculosis. Table II gives the results of our observations.

In Table II the first three cases show what we would consider a normal distribution between the young and the segmented forms of the neutrophile. These cases agree with the findings of Schilling who has established that about 4 per cent of the total leucocytes are of the "band" form. We would consider that the nonsegmented forms might be as high as 7 or 8 per cent without real pathologic significance. If the immature forms are maintained above 8 per cent, the neutrophile count is abnormal and the greater the percentage of nonsegmented forms the more serious is the pathologic condition.

Case G E (acute appendicitis) shows that although there was a mild leucocytosis and a high percentage of neutrophiles the nonsegmented forms were not increased above normal. Operation was performed within twelve hours. Histologic examination of the appendix showed extensive neutrophilic infiltration of the tissues with but little exudate free in the lumen of the organ.

Case E S shows a normal total leucocytic count except on the day of death when a marked leucocytosis was present. Note the marked and persistent shift in the neutrophiles to the nonsegmented forms. The count done on the day of death showed 70 per cent of all leucocytes to be nonsegmented neutrophiles. This case, as well as several others in the table, agrees with the observation of Schilling that the total leucocyte count gives much less reliable information than the differential leucocytic count, if the latter is properly done and correctly interpreted. One might be led to the conclusion that the bone marrow in the case was incapable of causing a leucocytosis, that it was exhausted. That this was not true is shown by the agonal leucocytosis with a tremendous outpouring of immature forms. Myelocytes were frequently present but it was not necessary to have a greater subdivision of the neutrophile types to give a clear understanding of the seriousness of the pathologic process.

Case L B demonstrates that a decided shift to the immature neutrophiles occurs during the height of a positive smallpox vaccination reaction.

TABLE II

CASE	PARAMETER	DATE	TOTAL COUNT	NO. OF SPOTS	NO. OF SPOTS PER UNIT	NO. OF SPOTS PER UNIT	NO. OF SPOTS PER UNIT	NO. OF SPOTS PER UNIT
Cn	No disease	7/11	5 100	5	50	7	0	0
		8/11	6 100	1	72.5	1	0	0
		8/11	7 700	7	41	10	1	1
		8/12	6 600	1	15	10	1	1
		8/15	1 500	1	16	10	0.5	1.5
Pw	Malnutrition	7/20	6 400	1.5	51	7	1	1
		8/12	8 200	7	55	6	0.5	0.5
		8/15	9 900	1	55	7.5	0	0
		8/25	7 700	7	56	8	1.5	1.5
		6/20	6 700	1	49	7	0.5	0.5
VL	Incipient pulmonary tuberculosis	8/7	7 000	1	52	12.5	0	0
		8/11	8 100	1	56	12.5	0.5	0.5
		8/21	8 200	7	51.5	10	0.5	0.5
		7/26 AM	11 000	8	82	7	0	0
		7/26 PM	11 500	7	80	1.5	0.5	0.5
GE	Acute appendicitis (normal convalescence after operation)	8/6	7 400	7	70	1	1	1
		8/28	5 600	7	61	11	1	1
		6/17	5 100	33	50	7	1	1
		6/19	6 600	13	44	1	0	0
		6/21	8 200	21	35	1	0	0
PS	Streptococcus viridans Indocavititis with septicaemia Died August 2	6/21	9 000	17	38	0	0	0
		7/28	6 600	13	44	1	0	0
		7/30	8 000	15	59	7	0	0
		8/2	23 900	70	20	1	1	1
		12/1	6 100	12	44	21	2	2
LB	Chronic bronchitis Positive "Poke" test following smallpox vaccination	12/5	5 700	23	42	1	1	1
		12/8	7 000	17	40	13	0.5	0.5
		12/9	6 600	25	37	18	0.5	0.5
		12/12	7 100	11	41	1	0.5	0.5
		12/15	8 600	6	46	12	0.5	0.5
		12/18	7 600	8	55	7	1	1
		12/22	6 800	9	49	15	1.5	1.5
		12/23	4 600	8	49	14	1	1
		12/26	5 700	8	51	15	0.5	0.5

TABLE II—CON.F'D

CASE	REMARKS	DATE	TOTAL COUNT	NEUTROPHILES		LYMPHO CYTES	MONO NUCLEARS	EOSINO PHILFS	BASO PHILFS
				NON SEG	SEG				
Ho	Carcinoma of lung	6/19	7 100	9	60	13	14	3	1
		8/ 4	6 600	7	63	14	11	4 5	0 5
		8/11	6 200	14	58	11	13	4	0
		8/18	6 700	10	62	13	12	3	0
		8/25	5 400	11	55	25	7	1 5	0 5
X	Incipient pulmonary tuberculosis	9/ 2	5 500	10	62	11	12	4	1
		6/23	5 800	6	54	30	8	1 5	0 5
		8/19	3 800	3	46	39	10	2	0 5
		8/26	3 900	4	44	40	10	1 5	0 5
		9/ 3	4 800	7	46	32	10	4	1
	Acute gastrointestinal upset due to m discretion in beverage	9/ 9	5 200	6	48	37	6	2 5	0 5
		9/11	10 000	21	66	7	5	0	1
		9/12	5 500	13	45	32	7	2 5	0 5
		9/13	3 600	8	40	37	12	3	0
		5/15	9 400	37	39	11	10 5	1 5	1
De L	Far advanced pulmonary tuberculosis Died Nov 20, 1930	5/16	14 700	30	45	9	11	1 5	0 5
		5/19	8 700	33	44	8	13	1	1
		5/20	12 500	31	53	5	10	1	0
		5/22	10 200	21	50	11 5	16	0 5	0
		5/23	10 000	27	54	6 5	12	0 5	0
		5/26	10 400	29	50	8	12	1	0
		5/27	9 900	33	47	6	12	1 5	0 5
		5/29	8 000	24	45	11	18	1 5	0 5
		5/30	11 200	30	47	11	10	1 5	0 5
		6/ 2	8 400	32	45	10	12	1	0
		6/ 3	5 400	37	45 5	7	10	0 5	0
		6/ 5	8 000	38	41	6	13	1	1
		6/13	10 000	22	36	11	10	1	0
		6/16	14 900	24 5	50	10	11	4	0 5
		6/17	12 000	30	46	11	10	3	1

TABLE II—CONT'D

CASE	REMARKS	DATE	TOTAL COUNT	VEGETOBLASTS		LYMPHO CYTES	MONO NUCLEARS	EOSINO PHILES	BASO PHILES
				NON SFG	SFG				
Sch	Moderately advanced pulmonary tuberculosis. Has a large basal lesion. Patient has shown considerable clinical improvement during sanatorium residence.	5/12	10 500	11	51	22	12	3	1
		5/15	9 300	12	51	19	14	3	1
		5/16	6 400	8	54	20	14	3	1
		5/19	8 500	10	50	23	13	3.5	0.5
		5/20	9 000	9	52	22	14	2.5	0.5
		5/22	9 000	4	53	25	15	2.5	0.5
		5/23	8 700	7	50	26	12	4.5	0.5
		5/26	9 000	6	55	20	15	3	1
		5/27	9 500	10	51	23	13	2.5	0.5
		5/29	8 000	7	55	21	12	4	1
		5/30	6 800	9	51	23	13	2.5	1.5
		6/2	7 100	9	49	22	13	6.5	0.5
		6/3	6 900	10	49	23	13	4	1
		6/5	9 100	9	53	24	11	2.5	0.5
		6/13	9 400	7	57	19	11	5.5	0.5
		6/16	9 600	8.5	54	23	9	5	0.5
Bo	Far advanced pulmonary tuberculosis. Artificial pneumothorax, pneumolysis and phrenectomy.	6/17	5 800	8	55	20	12	1	1
		7/29	8 100	6	60.5	24	8	0.5	1
		6/5	7 300	5	60	28.5	8	1	0.5
		6/19	9 600	4	58	32	4	2	0
		6/24	7 400	4	57	31	6	1.5	0.5
		7/31	9 000	7	64	19.5	8.5	1.0	0
		8/7	8 300	16	56	14	13	1	0
		8/1	17 000	8.5	66	16	9	0.5	0
		8/8	15 000	12	70	10	8	0	0
		8/15	11 000	15	62.5	13	9	0	0.5
Sw	Far advanced pulmonary tuberculosis. Artificial pneumothorax. Very little clinical improvement.	8/22	12 300	10	71	11	9	0	0
		9/5	19 000	12	69	12	7	0	0
		9/30	16 500	9	70	10	9	1.5	0.5
		10/28	15 300	13	66	13	7	0.5	0.5

TABLE II—Cont'd

CASE	REMARKS	DATE	TOTAL COUNT	TUBERCLES		TUBERCULIN UNIT	NO. OF TUBERCLES	POST-OP TUBERCLES	BASO- PHILS
				NO. OF TUBERCLES	STAIN				
Br	Moderately advanced pulmonary tuberculosis. Artificial pneumothorax. Clinical symptoms improved	5/27	7,600	7	60	17	0	0	0
		6/1	21,000	12	14	11	12	1	0
		6/16	7,400	8	70	20	17	3	0
		6/23	12,000	11	61	15	0	6	1
		7/30	8,900	17	78	15	7	1	0
		8/1	6,100	11	74	20	11	3	0
		8/11	8,500	12	51	18	8	1	0
		9/1	8,400	11	64	11	8	1	1
		9/30	9,100	6	62	17	11	1	1
		8/6	7,800	5	56	20	7	1	1
Stc	Moderately advanced pulmonary tuberculosis. Artificial pneumothorax. Good clinical progress	8/11	9,000	5	60	14	8	1	1
		8/20	6,600	5	74	10	8	3	1
		8/27	8,500	4	60	37	10	1	0
		10/1	7,100	1	56	28	0	2	0
		10/6	9,500	6	60	22	10	1	1
		10/11	8,700	7	78	22	11	1	1
		10/20	10,500	7	78	23	10	1	1
		10/28	10,000	2	40	17	0	1	1
		5/30	10,200	2	71	15	8	1	0
		6/6	4,500	2	51	74	0	2	1
Sta	Far advanced pulmonary tuberculosis. Artificial pneumothorax. Excellent clinical progress	6/11	7,100	1	51	12	10	1	1
		6/20	5,800	1	60	32	8	1	0

This promptly receded with the subsidence of the pathologic process. Here again the differential count gives information which would be missed entirely if the total count alone was relied upon.

Case Ho demonstrates that the shift in neutrophilic forms can occur in malignancy. Again a normal total count is present.

Case X shows that this same shift toward immature neutrophils can occur when there is no evidence of infection. The significance of this will be emphasized in the discussion. In this case a total leucocyte count of 10,000 is evidence of a leucocytosis for he normally runs between four and six thousand in his routine counts.

Case De L was a case of extensive and progressive pulmonary tuberculosis who had been seriously ill for three years before death. Considerable variation occurs in the number of immature neutrophils from time to time but they are always well above the normal percentage. Marked fluctuations also occur in the total counts. There is no correlation between the total leucocyte count and the percentage of immature neutrophils.

Case Lo was very similar to Case De L. The percentage of mature forms of neutrophils ranges higher and the immature forms lower than in Case De L. Note the marked increase of immature neutrophils at the time of the acute inflammation of the facial tissues and the prompt subsidence following extraction of a diseased tooth. Although the immature forms are less in this case than in Case De L the percentages of all neutrophilic forms are about the same in both instances. Death occurred sooner in this case than in Case De L.

Case Ca is another serious case of tuberculosis, the patient having been bed-ridden for over three years. Note the fluctuations in the nonsegmented forms and the tendency to maintain a high level in segmented forms of neutrophils.

Cases Re and Dic are included to show that a marked increase of nonsegmented neutrophils occurred with a clinical exacerbation of the tuberculous process. Note that this increase has persisted since the first rise was observed. In case Dic this increase in immature forms antedated the clinical exacerbation of the disease.

Case Sch shows that a case with a large basal pulmonary lesion need not exhibit a constant shift in the neutrophilic forms to the nonsegmented types. This is of real significance since it informs one that the neutrophils are not in excessive demand.

Case Bo is included to illustrate the effect of increased physical activity upon the leucocytic reaction. This is a case in which artificial pneumothorax, pneumolysis and phlebotomy failed to control the progress of the disease. The last two counts were done just prior to her discharge from the Sanatorium and show a marked rise in immature neutrophils on the last count.

In case Sw there has been a persistent leucocytosis. The neutrophils were increased above normal in both the nonsegmented and segmented forms. Even where there is a constant leucocytosis the greatest increase of immature neutrophils does not occur with the highest total count. Artificial

pneumothorax has not changed the leucocytic picture and thus indicates the inefficiency of the treatment in this particular case.

Case B₁ is quite similar to Case S_w except that here we do not have a persistent leucocytosis.

Cases S_{te} and S_{tr} are two individuals in whom artificial pneumothorax has brought about a very marked shift in the differential leucocytic picture from the neutrophile toward the lymphocyte side. Such a change was noted in doing the differential counts in the usual way without separation of the neutrophiles into distinct groups. At the time the counts included in Table II were done both cases were improved clinically. The leucocytic picture taken as a whole is consistently better in Case S_{tr}. In neither case is there an increase in nonsgmented neutrophiles.

DISCUSSION

Parley et al. state that "the test of a given clinical procedure is often the ease of its application divided by its usefulness." We wholly agree with this statement. Any laboratory method which can be of routine value should be reduced to the minimum of work consistent with giving as much information as possible. In special instances an elaboration of the simplified method may be justifiable but it is doubtful whether the added information gained will render a much more accurate insight into the biologic process being studied. While one always desires scientific data to be minutely accurate still in biologic processes one is interested more in the limits of variation which may be considered within normal and in the significance of the variations beyond normal limits. In any instance one should not be too dogmatic about the limits set for normal. It should be recognized that fluctuations above or below normal will occur which may have no clinical significance. It is only when such fluctuations beyond a normal range persist that serious attention should be given them. From this one can readily see that a single differential leucocytic count is insufficient. With a simple technique more counts and hence more information may be gained with the same amount of labor used in a more elaborate method.

Most physicians are interested in leucocytic counts as diagnostic aids in infectious processes. We are of the opinion that the leucocytic response in pathologic processes extends far beyond the domain of infections. While abnormal leucocytic reactions are found consistently in infections of any severity, it should be borne in mind that such a finding is but one phase of a general biologic rôle played by the various leucocytes. Noninfectious lesions, such as infarction of heart muscle, severe burns, intraabdominal hemorrhage, etc., may cause as marked an alteration in the leucocytic picture as an infectious process. It would seem more logical to regard a leucocytic response of whatever type as indicative of the type of alteration in the tissues to which the various leucocytes respond than to insist that an infection must be present whenever an abnormal leucocytic picture is encountered.

We have included Case X in Table II to show that one may encounter a marked increase of immature neutrophiles as a response to a noninfectious lesion. The illness in this case was the result of the indiscreet choice of an

alcoholic beverage To us this case demonstrates the fallacy of attempting to determine the presence or absence of an infection by the leucocytic response found That the leucocytic picture will be of great aid in determining the severity of the process, whether infectious or noninfectious, is unquestionable The prompt return of the leucocytic formula in this case to normal was quite striking

There is quite a tendency among physicians to rely more upon the total than upon the differential leucocytic count The cases included in this paper show that one cannot rely upon the total count alone as an index of an abnormal leucocytic picture A mild leucocytosis may occur with a normal differential count or one may encounter a marked shift in the numbers of the different cell types with a normal or even a low total count If it is possible to have only a total or a differential count then it is better to choose the differential count to obtain information However, both the total and differential counts should be obtained wherever possible for together they give more information than either alone

The classification of the leucocytes as stressed by the authors mentioned in this paper has had a tendency to emphasize very strongly the neutrophile and to lay less stress upon the other cell types This is commendable to a degree for in those pathologic processes most incompatible with life of the tissues the neutrophile plays the leading rôle However in evaluating any leucocytic count one should always note the proportions of the different cells, for each type has its own significant part to play This can best be appreciated by comparing the counts in Case De L with those in Case Sel In previous papers⁷⁻⁹ we have dealt in detail with the significance of the different leucocytic types in tuberculosis and it would seem to be unnecessary repetition to comment further upon this subject here

During the past several years we have made a very extensive study of the leucocytes in various diseases We have used a classification in which we did not attempt to divide the neutrophiles into nonsegmented and segmented forms although we had noted an increase of the nonsegmented forms very commonly in patients who were seriously ill From our present study we are led to the belief that it is hardly necessary to have the elaborate subdivision of the neutrophiles if one appreciates fully the normal mode of the leucocytic types Another factor which has come to our attention is that one must not place too great importance upon the extent of the shift in the neutrophile types It will be noted that in our cases there has occurred marked fluctuations from day to day The significant and very important point is that in the patients who were seriously ill the nonsegmented neutrophiles were consistently above normal

If one considers the presence and the extent of the shift in the neutrophilic forms as more significant than the total number of all neutrophiles then erroneous conclusions may be drawn For instance Case G E had practically no increase in nonsegmented forms although her total neutrophiles were markedly increased To have awaited a shift in the neutrophilic types before operation was advised would have in this case caused an inexcusable delay

It will be noted in some of the cases included in Table II that there is

but little if any difference in the total number of neutrophils although there is considerable difference in the number of nonsegmented forms (Compare Cases De I, Lo, and Ca). We have observed that in a number of serious tuberculous cases there occurred an abnormally high percentage of segmented neutrophils with only a slight to a moderate increase of nonsegmented forms. To conclude that those cases with a high percentage of nonsegmented forms are more critically ill than those with a moderate increase would be erroneous as is shown in Cases De I and Lo. It would appear probable that in some individuals there has occurred a sufficient expansion of the hematopoietic tissue in the bone marrow so that mature neutrophils can be produced rapidly enough to meet the demand and hence one does not find a high percentage of the immature cells in the circulation. From our study it seems to us that the shift in neutrophilic types signifies more the ability of the marrow to produce mature neutrophils in sufficient number than it does the seriousness of the pathologic process. In acute pathologic conditions such as lobar pneumonia the sudden demand for neutrophils does not allow time for the hematopoietic tissue to sufficiently expand to meet the demand with mature cells hence a very high percentage of nonsegmented neutrophilic types in the circulation. In chronic progressive tuberculosis there is time for the expansion of the hematopoietic marrow tissue and if this expansion has occurred one may find only a slight increase of the immature forms.

In the Schilling method great importance is placed upon the presence of the most immature neutrophils viz. the metamyelocyte and the myelocyte. Wherever there is an excessive demand for neutrophils one would expect a few of the most immature forms to be present in the circulation. In any cell type, where great pleomorphism is shown in the developmental cycle, too much stress should not be given to any one phase of the process. We agree with Pincus that Schilling's Classes I and II may very readily be included in his Class III for routine clinical work. The same may be said of Class I of Pons and Krumbhaar.

A criticism that may be made relative to differential leucocyte counts as done in a large number of places is that but one hundred leucocytes are counted. The author considers this an insufficient number, for no matter how expertly the blood films are made the different leucocyte types will not be evenly mixed. It is probable that they are not evenly mixed in the circulating blood. We feel that four hundred cells should be counted in every differential count in order that the percentage of the leucocyte types of smaller numbers may be more nearly correct. If a high dry magnification of four hundred is used one may classify four hundred cells as accurately and almost as rapidly as one hundred under oil immersion.

Failey, et al., state that as high as 16 per cent of nonfilament neutrophils may be regarded as normal. If such be the case then a very high proportion of our counts cannot be considered pathologic. Since 65 per cent of their normal counts have 10 per cent or less of nonfilament neutrophils we believe it justifiable to consider the remainder abnormal. It is not uncommon to find single abnormal counts in individuals who are considered healthy. Here we wish to stress again, as we have in a previous paper² that one must

not place too great reliance on a single leucocytic count. The persistence of abnormality in repeated counts is the thing of real significance.

SUMMARY

We believe that, if one desires to divide the neutrophils into different groups in the differential leucocytic count it is sufficient for practical purposes to establish but two classes, nonsegmented and segmented. Such a classification often gives more striking proof of the abnormality of the leucocytic picture than the grouping of all neutrophils together. However in the majority of instances, one need not have this subdivision of the neutrophils to appreciate the fact that an abnormal leucocytic reaction is present.

We consider, from our studies, that a persistence of more than 8 per cent of nonsegmented neutrophils is abnormal. The sustained increase rather than the increase in a single count is the point of importance.

To attempt to diagnose infections by any leucocytic count we regard as fallacious, for noninfectious tissue lesions can cause a leucocytic reaction identical to that caused by an infectious agent. In either instance the leucocytic response simply indicates the type and severity of the tissue damage.

In many instances an abnormally high percentage of neutrophils occurs without any or with but slight increase in nonsegmented forms. Such a leucocytic reaction should be given as careful consideration as instances where there is a marked shift to the nonsegmented forms. This reaction we have observed in early acute appendicitis, proved by operation, and in chronic progressive tuberculosis. It would seem that the shift to immature forms depends very largely upon whether the amount of leucopoietic tissue is sufficient to produce sufficient segmented mature neutrophils to meet the demand. The change in the neutrophilic forms may indicate the volume of leucopoietic tissue the individual case possesses more than the seriousness of the pathologic process.

We regard single leucocytic counts and the counting of but 100 cells in the differential as insufficient in any case where leucocytic studies are deemed of value.

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A SIMPLE THERMOCOUPLE AND A THERMOPILE FOR DETERMINATION OF TEMPERATURE IN BIOLOGY AND MEDICINE*

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THE thermocouple is a very sensitive type of thermometer and with proper care will indicate minute changes in temperature quickly and accurately. It is adaptable also to various special requirements of a problem; for instance, it can be made either flexible or rigid, and it is possible to make the sensitive point very small and insert it like a needle into tissues. The temperature of situations difficult of access can often be determined with the thermocouple and readings taken at any desired distance away.

An additional feature which is sometimes valuable is that any number of individual points or junctions can be connected to the main thermocouple and used in various locations at the same time. A continuous record of temperature is possible with the aid of accessory apparatus.

The thermocouple is comparatively easy to make and equally simple to use. If the details of construction and operation were more generally known this excellent method of reading temperature might be more widely used. Following is a description of the construction and use of a thermocouple and a thermopile and of the principles involved in the method.

THE PRINCIPLE OF THE THERMOCOUPLE

If wires of any two dissimilar metals, copper and nickel for instance, are fused or soldered end to end, a 'thermoelectric' junction will be formed at each connection. A thermocouple consists essentially of two such junctions connected into a circuit, as shown at *A* and *B* in Fig. 1a. The junctions are called thermoelectric partly because one of the dissimilar metals has a greater number of electrons than the other or the electrons have greater energy values, and consequently a flow of electricity will take place from one metal to the other. This results in an "electromotive force" (e.m.f.) being set up at each junction, and the magnitude of this force depends upon the temperature of that junction.

The electromotive forces generated at the two junctions of the thermocouple are opposed to each other; consequently if the temperatures of the junctions are the same the forces will be balanced and no current will flow.

However, if one temperature is higher than the other, there will be an excess of e.m.f. at the warmer junction, and the result will be a flow of current around the thermocouple which would cause a deflection in a galvanometer. The energy necessary to maintain this current is derived from the additional heat absorbed at the warmer junction.

*From the Otho S. A. Sprague Memorial Institute and the Department of Pathology of the University of Chicago.
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A current in the thermocouple, therefore, depends upon a difference in temperature being established between the two junctions. The "cold" junction is kept in a known and constant temperature as in a mixture of ice and water in a thermos bottle and the "warm" junction is placed in contact with the material of unknown temperature. Since one temperature is known and since the e m f generated varies directly with the temperature difference, the unknown temperature can readily be determined.

THE PRINCIPLE OF THE POTENTIOMETER

In actual practice it is a simple matter to determine the unknown temperature quickly when the apparatus is set up. However, it is well to know why certain instruments have to be used. The e m f generated in the thermocouple can be measured in two ways by the galvanometer and the potentiometer methods. The former measures the current directly and is the simpler one. However, it involves certain errors due to the resistance of the galvanometer in the circuit and also of the leads which are the copper wires connecting the junctions with the accessory instruments. This resistance varies with the length and diameter of the leads and especially with differences in temperature. True it is possible to counteract this by introducing into the system an equal and opposite resistance, but the conditions causing variations in the resistance of the circuit have to be known and carefully standardized to obtain comparable results at different times.

Fortunately these resistances may be disregarded by the use of a potentiometer. This is an ingenious device by which only the e m f of the circuit and not the current, is measured. No current flows at the time the measurement is made, consequently resistance ceases to be a factor, and the leads may be of any length and diameter and may pass through any variations of temperature without affecting the accuracy of the readings.

When a temperature is to be read, the e m f of the thermocouple circuit is simply opposed by balancing against it a difference of potential supplied through a secondary circuit. When the two circuits are completely opposed there can, of course, be no flow of current in the system, and the galvanometer will stand at zero. The reading of the potentiometer is taken at that time and will show the magnitude of the e m f drawn from the battery in the secondary circuit to balance that of the thermocouple. The reading of the potentiometer is divided by a value (obtained by calibration as described later) and the result will indicate the unknown temperature.

CONSTRUCTION OF THE SIMPLE THERMOCOUPLE

Any of the various types of thermocouples required by the biologist can easily be made in the laboratory. The two metals recommended are copper and constantan. The latter is an alloy of 60 per cent copper and 40 per cent nickel. Wire of these two metals may be purchased in a number of gauges and with silk, cotton or enamel insulation. Wire of small diameter such as B & S No 34 gauge is recommended, and double silk covered insulation is generally preferable. It is advisable to coat the wire with shellac before using, to strengthen the insulation and to permit easy removal at the points without fraying.

A length of constantan wire, equal to the distance between the material of unknown temperature and the location of the thermos bottle is taken, also a length of copper wire about 2 feet longer. The insulation is very carefully removed with a sharp knife from both ends of the constantan and from one end of the copper wire the precaution being taken not to scrape the metal unduly with the knife. The copper is connected to one end of the constantan by winding the ends together to form a spiral as in Fig. 1 b. This junction is next evenly soldered. A good method is to melt a little solder upon a clean surface of a soldering iron and after applying the flux to immerse the junction in the hot metal from *C* to *P* Fig. 1 b. This is to be the warm junction

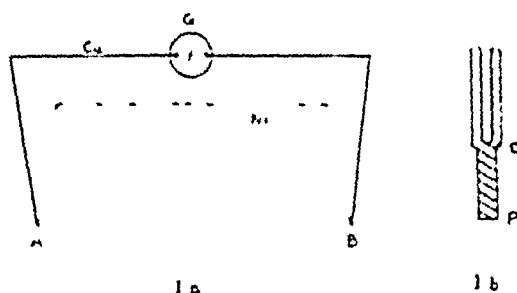


Fig. 1 a—A thermocouple circuit. 1 b—A thermoelectric junction in cross section.

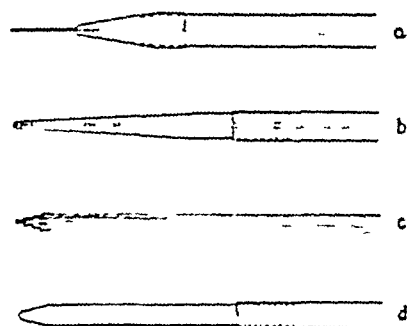


Fig. 2—Different methods of finishing the thermocouple and thermopile junctions enlarged.

The other end of the constantan wire is soldered in a similar way to a piece of copper wire about 2 or 3 feet long, to form the cold junction.

To prevent tangles of the wire and wear upon the insulation it is well to enclose the body of the couple in a light rubber tube, leaving the junctions exposed. If instantaneous readings of temperature are required, it will be sufficient to coat the warm junction end with a protective surface such as a waterproof varnish or a lacquer and fasten the rubber tube down near the end with adhesive tape as in Fig. 2-a. If a momentary lag is permissible better results will probably be obtained by enclosing the warm junction in a very small, thin-walled glass tube with one end sealed as shown in Fig. 2-b. The junction is first dipped in melted paraffin and, while the wax is still soft, is pressed to the extreme tip of the tapered tube. This will exclude air and greatly reduce lag. Warming the tube will assist in getting the junction to

the tip. The open end of the glass tube is plugged to hold the couple in place. The rubber tube overlaps the glass slightly and is held in place with adhesive tape. Suitable glass tubing may be made by drawing out a test tube over a gas flame.

It is important to make the junction *CP* Fig 1-b, as short as is consistent with strength, probably about 2 mm. in length. Also when making a determination the junction should always be inserted into the material beyond the point *C* preferably to a uniform depth of immersion.

The cold junction which is to be placed in a thermos bottle should be enclosed in a length of glass tubing sealed at one end, and allowed to rest in the ice water 2 or 3 inches. A convenient exit for the lead wires is between the glass and rubber tubing at the cold junction end. These wires are the two which connect the couple to the potentiometer, shown in Fig 3.

SINGLE AND MULTIPLE THERMOCOUPLES

For the determination of one unknown temperature the arrangement shown in Fig 3 will probably be satisfactory. "*A*" is the warm junction and

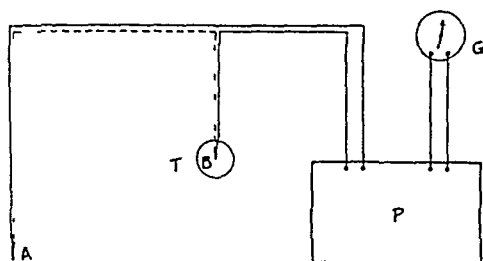


Fig 3—A set-up for a single thermocouple and also for a thermopile.

is to be placed in contact with the material of unknown temperature, "*B*" is the cold junction in the thermos bottle "*T*". The copper wires leading from the 2 junctions are connected to the potentiometer "*P*". The galvanometer "*G*" indicates when balanced conditions have been obtained. The distance from *A* to *B* may be any length required. The constantan wire in all the diagrams is represented by a broken line and the copper by a full line.

Occasionally it is necessary to determine 2 or more temperatures in quick succession or without removing the points. It is quite feasible to do this and to take readings over an extended period, with only a slight modification of the couple. A multiple couple is used for this purpose, and one type is shown in Fig 4. It may have as many junctions as desired, each one being connected to the main constantan line by a branch of the same metal. The branches may be soldered to the main constantan wire at any convenient place. A detail drawing of the constantan connections is shown in the diagram "*C*" in Fig 4. Each junction is then numbered and a full length of copper wire is run to a similarly numbered post on the selector switch "*S*".

Maintenance of the cold junction temperature and the method of calibrating the couple are described later in the paper.

THE THERMOPILE OR COMPOUND THERMOCOUPLE

Readings of temperature within 0.01 or less is sometimes necessary. It is seldom practicable to attempt to read this closely with the single couple especially if used in connection with a galvanometer of ordinary low sensitivity. Large deflections in the galvanometer with small temperature changes may be obtained with a thermopile. This device is an adaptation of the principle of the thermocouple and a diagram of its arrangement is shown in Fig. 5. It consists of a number of single couples in series. Each copper-constantin couple will develop between 37 and 40 microvolts per degree difference in temperature of the 2 junctions. When a number of couples are placed in series an additive effect is produced and the total emf developed in the thermopile system may be comparatively large, depending upon the number of couples used.

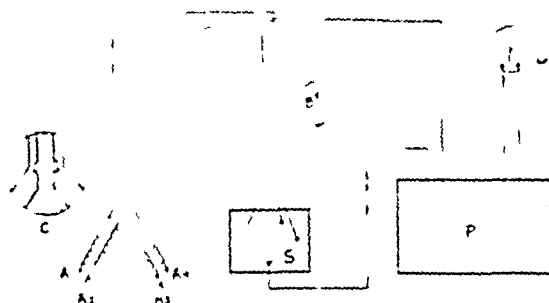


Fig. 4—Arrangement for multiple thermocouple

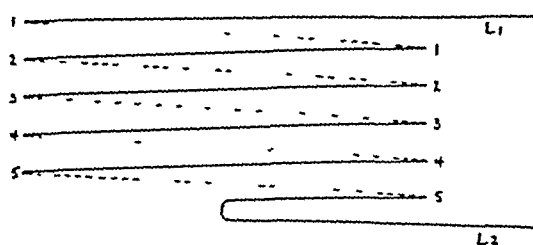


Fig. 5—Diagram of a thermopile system

CONSTRUCTION OF THE THERMOPILE

A board about 3 inches greater than the required length of the thermopile is obtained and close to each end a row of short nails is driven in about one-quarter inch apart. There should be 2 nails for each junction plus an extra one at each end for the second lead wire. That is for a thermopile of, say 15 junctions 31 nails would be used at each end.

Wire of small diameter such as B & S Nos. 34 or 36 gauge is recommended and double silk covered insulation is very satisfactory, although enameled wire may have an advantage where the diameter of the bundle is to be reduced as much as possible. Each odd-numbered nail at one end of the board is connected to the corresponding nail at the other end with a length of copper wire, and each even-numbered nail is connected in a similar man-

ner with constantan. All the wires are next given a coat of shellac to strengthen the insulation and to permit its removal at the tips without fraying. Adequate insulation is important and at this stage much trouble may be prevented.

When the shellac is dry, the wires are fastened down to the board about 6 inches from each end with a strip of adhesive tape. Then with a pair of scissors the wires are cut from the nails.

There is now a number of pairs of copper and constantan wires of equal length lying parallel in preparation for soldering. With a sharp knife the insulation is very carefully removed to expose about one-half inch of metal at each end. Emphasis must be placed upon the need of doing this with caution to avoid scraping the metal unduly. Each pair of wires is next connected by twisting to form a spiral as in Fig 1-b. The wires must be so joined that a continuous connection is formed with all the junctions in series as shown in Fig 5. The junctions are then soldered, and an electric soldering-iron is preferable as it remains hot while in use. The iron should have a flat surface, and upon this some solder is melted. After fluxing, each junction is immersed for an instant in the hot metal which should be deposited in a thin even covering upon the wires.

The points are next cut back to a uniform length of about 2 mm. At this stage it is advisable to test each point to be sure that it constitutes a thermoelectric junction. This is done by connecting to a galvanometer the 2 lead wires marked L_1 and L_2 in Fig 5 and touching each junction in slow succession with the warm fingers. A deflection indicates that the junction is in effective working condition. The tests should be made in a room free from sudden changes in temperature, otherwise there may be difficulties in controlling deflections due to variations in temperature of the 2 ends of the thermopile. A good plan is to make up each end of the thermopile into a temporary bundle but bending each junction away from the others so that none are touching. Then one bundle is placed in a wide test tube while trying out the other. Precaution should be taken when testing to avoid breathing upon the junctions.

The next step is one that requires especial care, namely, the complete insulating of the exposed metal of each junction. Unless this is done adequately the thermopile will not be satisfactory. For this purpose collodion has been found to give excellent results. Each junction is dipped into collodion beyond the exposed metal and allowed to dry thoroughly. Three coats are advisable to ensure a complete covering.

After thorough drying for several hours, the junctions at each end are made up into a permanent bundle. The points should each have an outside position. This can be done by placing 2 or 3 points at the tip and arranging the others closely together around the sides as in Fig 2-c.

Several methods of finishing the thermopile are available. It may be enclosed in a glass tube, which has some advantages for use in physical chemistry but is too rigid for many purposes in biology. A satisfactory enclosure can be made with rubber tubing. This does not interfere with flexibility and

gives sufficient protection from injury and short circuits. Thin walled glass tubing sealed at one end may be used at the warm end as shown in Fig. 2 d. The bundle of junctions is dipped in melted paraffin and pushed to the tip of the tube as described for the simple thermocouple. The bore of the tube should be as small as possible. If instantaneous readings are essential, the warm junctions should not be enclosed in glass but be dipped 3 or 4 times in shellac or other suitable material to protect the collodion. This outer covering must not of course be soluble in ether otherwise the insulation would be ruined.

The connection of the thermopile with the potentiometer is similar to that of the simple thermocouple and is shown in Fig. 3.

MAINTENANCE OF THE "COLD JUNCTION" TEMPERATURE

The temperature indicated by the thermocouple is as explained in the section on principles the difference between the temperatures of the 2 opposite junctions such as between *A* and *B* Fig. 3. One of these temperatures

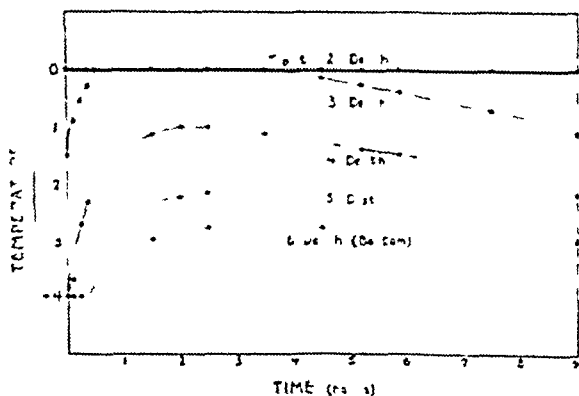


Fig. 6—Showing fluctuations in temperature in a thermos bottle when not well filled with ice.

that at *B* is required to be known, and the dependability of the couple as a precision instrument can be no greater than the accuracy with which the cold junction temperature is known. The simplest way to satisfy this requirement is to maintain the temperature uniformly constant.

Fortunately a constant temperature can readily be obtained in a suitable mixture of ice and water in a thermos bottle. The bottle should be made up with as much chipped ice from distilled water as it will hold and then be filled up with cold distilled water. Such a mixture will maintain for hours a temperature in the upper part of the bottle which will not fluctuate from zero more than 0.001 to 0.002°. This uniformity cannot be depended upon unless the thermos bottle is well filled with chipped ice. A bottle half-filled with ice and the remainder with water would produce a temperature gradient as in Fig. 6. This chart shows that the required temperature of zero is obtained only in the upper 2 to 3 inches, below which the temperature is continuously changing. A cold junction resting below the 3 inch level would cause large errors in readings.

When determinations are made over long periods it is advisable to insert a precision or a Beckmann thermometer in the cork at the cold junction end, and to note when the temperature begins to rise. Both thermometer and junction should of course, rest at the same level in the ice water.

CALIBRATION

The number of microvolts which the thermocouple or the thermopile will generate per degree of temperature difference between the two junctions, now remains to be determined. This value is obtained through the potentiometer. The couple or pile is connected to the potentiometer and the cold junction placed in the thermos bottle. The warm junction is then exposed successively to 3 or 4 known temperatures. The number of microvolts generated at each temperature is divided by the number of degrees of temperature between the cold and warm junctions. The results should be practically a constant for all temperatures with which biologists are concerned, provided determinations closer than 0.01° are not required. If finer readings are necessary, certain precautions to be mentioned later have to be taken.

The accuracy with which the temperatures used in calibration are known will depend upon the precision required in the thermocouple. If an accuracy in reading within, say, one-tenth of a degree or less is satisfactory, the couple or pile may be calibrated by the use of water-baths, the water being stirred and the thermometer accurate to that extent.

For greater precision, fixed points in thermometry such as melting and transition points are commonly used. The following are suggested:

- | | |
|------------------------------------------|------------------------|
| 1 Transition point of manganous chloride | 58.09°C |
| 2 Transition point of sodium sulphate | 32.38 |
| 3 Freezing point of distilled water | 0 |

Transition points accurate to 0.01° may readily be obtained. For instance, with sodium sulphate, about 25 grams of the anhydrous form, Na_2SO_4 , are placed in a clean, wide test tube and moistened with distilled water. The temperature of the mass will then slowly rise to 32.38° . To prevent loss of heat when the water is added, the tube should be insulated and corked. The warm junction is pushed into the mass, and when the temperature ceases to rise and remains constant for a time the number of microvolts generated, as read on the potentiometer, should be noted. That value divided by 32.38 will give the constant sought.

In all subsequent work, for assurance that the couple or pile and its accessory apparatus are in reliable working condition a frequent check-up with a known temperature is recommended.

HOW TO APPLY THE CONSTANT OBTAINED BY CALIBRATION

Having determined the number of microvolts generated per degree of temperature difference, and having established it as a constant C , it is then possible to read any unknown temperature to approximately 0.01° . This is done, of course, by dividing the number of microvolts generated by the con-

stant. The value for C should be reliable through the range of temperatures used in calibration and probably for all temperatures in which biologists are interested.

PRECAUTIONS NECESSARY IF READINGS ACCURATE TO 0.001 ARE REQUIRED

When temperatures are to be read with this accuracy special care is necessary in both the construction and the use of the thermopile, and unless precautions are taken it is doubtful if the readings can be considered reliable.

1. The wire used sometimes has mechanical or electrical flaws and must be checked for 'homogeneity' or uniformity. This can be done in the laboratory by the method suggested by White¹ or wire already checked may be purchased from some of the dealers who supply wire for thermocouples.

2. The maintenance of the 'cold junction' temperature previously described is obviously of increased importance in precision work, for the accuracy of determinations can be no greater than the constancy of the known temperature.

3. The temperature cmf relationship no longer takes the form of a straight line when measurements as close as 0.001 are to be made. Therefore the factor which in determinations to 0.01 may be regarded as a constant, now increases slightly in value with the temperature. This departure from the linear relationship, however, may be adjusted by reference to the data given in the International Critical Tables, Vol. 1, pages 57-58.

4. The transition points used in calibration should be determined to the third decimal place. The two points previously suggested would therefore be

Manganous chloride	58.089° C.
Sodium sulphate	32.384

The transition points correct to the third place can be obtained only with material of exceptional purity such as after two or three recrystallizations. These and other transition points are given by Richards and Yngve.² The International Critical Tables, Vol. 4, page 6, list a number of transition points.

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A SIMPLE APPARATUS FOR CLEANING COVERSGLIPS*

BY HAROLD GORDON M.D., ANN ARBOR MICH

THOROUGH cleaning of coverslips is an important detail of the duties of the technician in laboratories of histology, pathology, and bacteriology, but as usually conducted by the manual method, it is a time-consuming and irksome task. The following apparatus has been designed to facilitate this work and can be set up readily wherever compressed air is available. Several weeks' trial in the Pathological Laboratories of the University of Michigan has demonstrated its advantages over the old method of cleaning coverslips by hand. Breakages are less frequent and several packages can be cleaned at one time. The minimum of handling further insures freedom from grease, an important factor in insuring firm adhesion of the sections where the albumin fixative method is employed. The superiority of the new method over the old, in maintaining adhesion between section and coverslip has been demonstrated by actual experiment.

APPARATUS

A glass cylinder open at both ends, 2 feet in length and $2\frac{1}{2}$ inches in diameter, is fitted at one end into a Buchner funnel of somewhat larger capacity. The cylinder is firmly sealed into the funnel with a paste of litharge and glycerin. After allowing the paste to dry, the union is permanent and leak proof. The cylinder is attached to a suitable metal stand and the tapering end of the funnel is connected to an air pipe by means of stout rubber tubing. The upper end of the cylinder is fitted with a rubber cork, pierced to allow the insertion of two glass tubes, each about 10 mm. in diameter. Experience has demonstrated the advantage of a double outlet in preventing the cork stopper from being displaced when the air current is turned on. Both tubes are bent in "U" fashion, with one limb of the "U" short, the other long (see Figs. 1 and 2).

METHOD

The cylinder is filled to about a quarter of its capacity with soap solution and the coverslips are placed in the solution. (The soap solution has been found to be superior to any of the usual acid cleaning fluids.) The rubber cork is replaced and the air current turned on. Four half-ounce packages of $\frac{7}{8}$ " coverslips (or more of the smaller size) and three packages of the larger sizes have been found to clean quite readily. The air is allowed to bubble through the soap solution, continuously agitating the cover glasses, for about ten minutes and then shut off. The coverslips are next rinsed in four changes of distilled water, each change being aerated for a few minutes to insure re-

*From the Department of Pathology, University of Michigan.

removal of every trace of soap and complete the separation of the coverslips. The water is next drained off and replaced with 96 per cent alcohol and air is again allowed to bubble through for five to ten minutes. The alcohol should be saved and may be used repeatedly. The coverslips are then transferred to a metal tray covered with paper towelling. The tray is placed on top of an incubator or oven and the coverslips are allowed to dry in a dust free atmosphere. It may be well to interpolate a warning at this point. The use of a wooden rather than a metal tray or dish is attended by some risk of fire as also is the insertion of the alcohol moistened coverslips inside

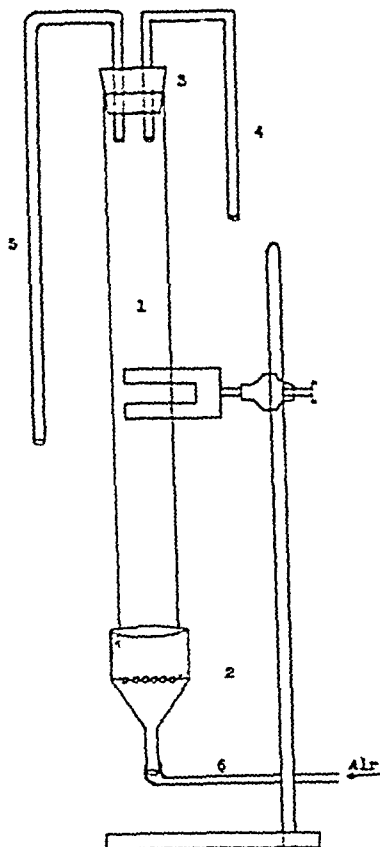


Fig 1—1 Glass cylinder for receiving coverslips. 2, Buchner funnel. 3 Perforated cork for exit tubes 4 and 5. (These can conveniently be twisted around to empty into a sink or basin.) 6 Rubber tubing connecting with compressed air line.

instead of on top of the incubator or oven. The actual washing of the coverslips can easily be accomplished in half an hour and while they take some time to dry, the interval may be utilized at other tasks since they require no further attention. Although simple in construction and principle, for the successful operation of the apparatus as outlined above certain precautions must be borne in mind. The soap solution should not have such a high content of soap as to render the solution too "bubbly" while aerated. A little experience will soon enable the operator to gauge the correct proportion of soap to use. An excess of soap causes the bubbles to escape by the

exit tubes the solution as a result either "boiling dry" or overflowing in a messy manner. The long ends of the exit tubes should be so arranged as to reach into a sink or a large basin. The air should not be allowed to enter too suddenly or forcibly and the exit should be sufficiently wide to make for a ready escape of the air. The double exit tubes are of special advantage where the water supply is free of hardness or chemicals since the rinsing

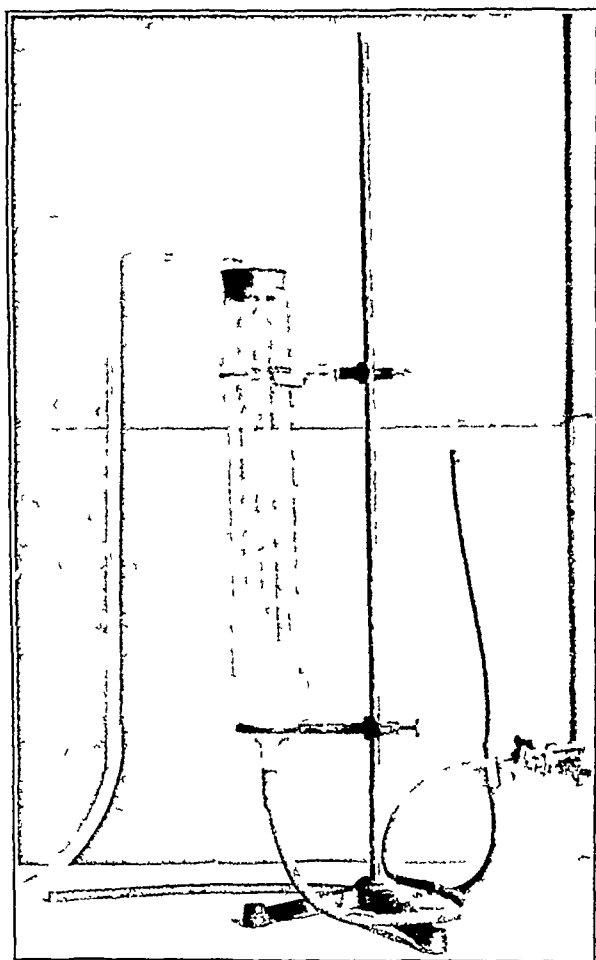


Fig 2—Actual photograph of apparatus. The two lines within the cylinder are due to reflections from its wall. The exit tubes end immediately beneath the perforated stopper at the top.

process may then very readily be effected by connecting one exit tube to the water tap and disconnecting the air-tube to allow the water to run in from above and escape from below the other exit tube allowing the air between these two channels to gain exit, thereby not disturbing the rubber cork. Finally, the rinsing must be thorough. The least trace of soap in the alcohol spoils it for subsequent use and causes the coverslips to be streaked. Complete removal of the soap insures beautifully clear and glossy glasses.

A NEW METHOD FOR STAINING BACTERIUM TULARENSE IN TISSUE SECTIONS*

BY LEE TOSKEY, M.D., CINCINNATI, OHIO

DURING the course of some work on experimental tularemia in animals I have found that several dyes are capable of staining the *Bacterium tularensis* in tissue sections in addition to Twort's light green neutral red mixture already used and reported by Edgingham.¹ A well differentiated Gram stain is unquestionably the best of all but there are other stains almost as good which are simpler of management and quite adequate for rou-

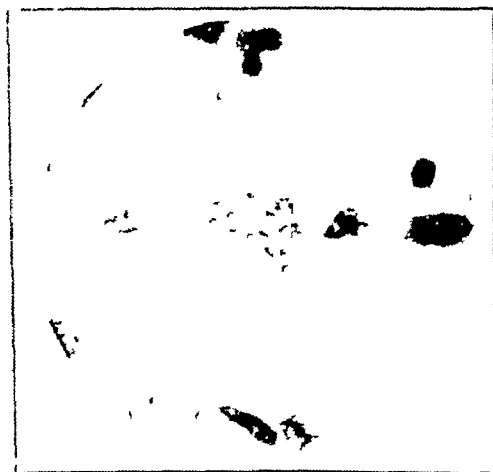


Fig. 1.—Photomicrograph of guinea pig spleen showing one large group and several smaller groups of *B. tularensis* living free in the intercellular spaces. Nile blue sulphate stain. Magnification 1550 Diam.

tine work. Of these stains the best in my experience, is Nile blue sulphate. When the bacteria are present in large numbers and arranged in fairly large masses in the tissues, as happens frequently in the spleens and lymphoid tissues of rodents and also occasionally in man, the dye may be used alone with good results. For routine work on animal tissues this is an adequate and rapid stain. The method is simple.

Deparaffinate the sections as usual and carry them through the alcohols to water. If heavy metal fixatives have been used, remove the metallic salts in the usual way. Stain in a saturated aqueous solution of Nile blue sulphate for from five to six minutes. Wash rapidly in water, dehydrate rapidly through the lower alcohols to absolute then carry through the xylols and mount as usual.

*From the Christ Hospital Institute for Medical Research.

When the bacteria are present in smaller groups they are somewhat difficult to find because of the relatively low contrast between the stained bacterial bodies and the cellular background. By diluting the stain and adding sufficient safranin to produce a purplish blue color, and using this mixture for overnight staining, a much higher color contrast is obtained. This renders the bacteria more readily visible and much easier to photograph. The method follows:

To 60 c.c. of distilled water add 10 c.c. of aqueous saturated Nile blue sulphate and 6 c.c. of 1 per cent aqueous safranin. When the deparaffinated sections have reached the water stage stand them in this solution to stain overnight. I have obtained good stains of certain tissues after only two hours in this solution but sixteen hours is better, although an hour or two more or less seems to make no appreciable difference. After staining wash rapidly in water and pass through the alcohols and xylols as usual.

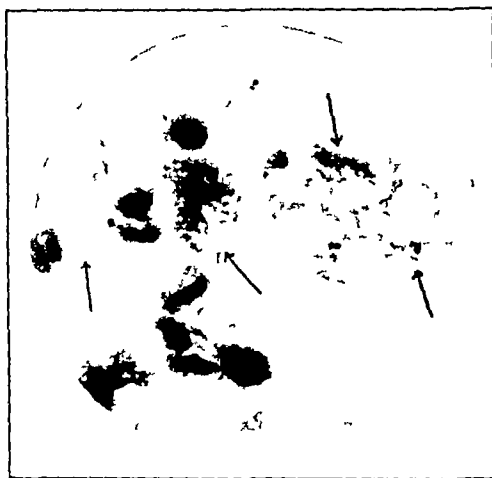


Fig. 2.—Photomicrograph of section of human lung (case of Bunker and Smith) showing both intracellular and extracellular groups of *B. tularensis*. Unstained sections from this case were very kindly supplied by Dr. C. W. McCoy, Director, National Institute of Health. Magnification 1550 Diam.

This method has been used to demonstrate the *Bacterium tularensis* in the lymph glands, spleen, liver, lung, kidney and skin of the guinea pig, in the lymph glands, lung, and spleen of the rabbit, in the lymph glands, liver, spleen, lung and kidney of the mouse, and in the lung from a fatal human case. This fatal case with necropsy findings is being reported in detail elsewhere. So far as I am aware it is the first time this organism has been demonstrated in human tissues.

The choice of a fixative solution for the fresh tissues seems to be a matter of no importance. When the bacteria are present they are well demonstrated by the Nile blue sulphate in tissues fixed in Zenker's, Orth's and Dommer's solutions, ethyl and methyl alcohols, and formaldehyde. There seems to be no difference in the tinctorial qualities following the use of any of these. When examining the mounted sections the use of an orange filter aids in giving added depth of color to the bacteria, thus increasing the con-

trast. As a matter of passing interest this stain also reveals the presence of a wide variety of bacteria in tissues. Pneumococci and streptococci are well shown in pneumonic lungs and in the smaller animals the typhimurium, bronchisepticus and leipsepticus are often encountered in various organs.

The color imparted to the bacteria is not a particularly good one for photographing. The addition of safranine produces a better shade for this purpose than does the Nile blue sulphate alone. A number of unsuccessful attempts have been made to improve on this.

A word is due regarding the finding of *B. tularense* in the guinea pig liver. I believe they have never been seen in this organ before. When normal guinea pigs are infected from cultures or from the spleens of animals dead of tularemia the survival period after infection is usually six days and four days respectively. Repeated examinations of the livers of such animals have consistently failed to reveal any bacteria either in or around the characteristic focal necroses. I have tried Giemsa stain, Twort's light green neutral red mixture as recommended by Ledingham, light green followed by safranine and Nile blue sulphate both alone and in combination with a number of other dyes. Bacteria are easily demonstrable in the spleens and in other organs but never in the liver. This was also Ledingham's experience.

The only guinea pig liver which has revealed the organisms in the focal necroses is the one from an animal that lived eleven and one-half days after infection from a culture. An effort had been made to immunize this animal by intraperitoneal injection of human convalescent serum. This did not protect but it did prolong life far beyond that of the other animals of the series and of the control animal.

SUMMARY

A method is presented for staining the *Bacterium tularense* in tissue sections. This method has revealed the organisms in human lesions from two different fatal cases and has demonstrated the organisms in the liver of one guinea pig.

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NONPOLARIZABLE ELECTRODES*†

BY JOHN W. WILLIAMS AND WALTER C. BOSCH, NEW ORLEANS, LA

A NONPOLARIZABLE electrode seems to some an impossibility. Nevertheless electrodes can be made which polarize less than those at present on the market. We have devised such an electrode which because of less heat production can be utilized in growing bacteria at more near then nor-

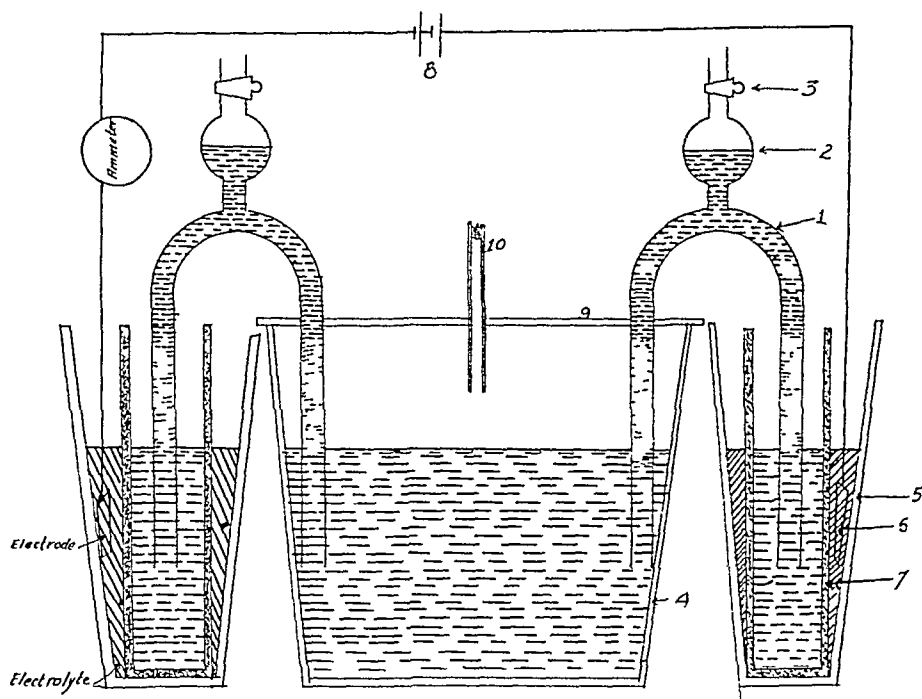


Fig 1

mal environs. By this means it is hoped an additional factor may be contributed to the classification of bacteria (i.e. a growth positive or growth negative organism) and that the lethal and optimum current for bacterial growth may be determined. In addition to the application of this device to bacteria it may be used in noting activities of inert particles. The media in which the bacteria must be grown are liquid since the electric current will disrupt the solid media and cause a short in the circuit.

Description of Device **Cross section** The device consists of a jar (4) in which the bacteria are to be incubated. This jar protected by a cover (9)

*From the Departments of Pathology and Physics, Tulane University.
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†Aided by a grant from the Schwartz Research Fund.

into the top of which a tube (10) projects. This tube is plugged with cotton and is for the purpose of injecting bacteria into the media. Like tubes may be placed adjacent to each electrode in order that bacteria may be drawn off from the poles whenever desirable. We note by the plate that ends of two electrodes project into this jar while similar ends of the same electrodes are received in porous cups (7). It will be noted by the line shading that the media in the electrodes, the jar and the described cups is the same (10) liquid media. On the top of each electrode will be noted a bulb (2) half filled with the liquid media. This media is capped by a negative pressure chamber resultant from the closed stopcocks (3). The procedure in effecting this fill is to open the stopcocks and fill the electrodes with the media from (4) and (7) by means of negative pressure and then close the stopcock. In this way should any fluid evaporate from the electric conduction system it would be replaced from (2) and there would be no interruption of current. The porous cups rest in a jar (5) filled with electrolyte in which electrolyte is the electrode (6) connected to direct current at (8).

This apparatus can be first filled and then sterilized. The porous cup may also be covered and in this way a further possibility of contamination avoided. The porous cup is so constructed that the electrolyte does not diffuse into it and contaminate the media. Care must be taken that the height of the electrolyte and media be kept the same and that both exert almost equal osmotic pressure. Although there will be slight change in the media in the porous cup as a result of fluid diffusion however sufficient diffusion will not take place to markedly influence an organismal growth so far removed as in this case. In the herein contained diagram the fluid levels are too high. It is far preferable that they be just above the electrodes.

A DIAGNOSTIC AID IN OBSCURE HEMATOLOGIC CONDITIONS*

By CARL REICH, A B, M D,† NEW YORK, N Y

THE hematologist is sometimes confronted with the difficulty of making a diagnosis in cases which appear to be in aleucemic phases of leucemia, but do not show sufficient numbers of pathologic cells in the stained films to permit a definite opinion. These patients usually present the picture of a severe anemia with leucopenia, and it is necessary to either await further changes in the peripheral blood picture or do a bone marrow biopsy.

The following method of procedure has shown itself to be of value and may at times obviate the necessity of a biopsy of the bone marrow. Ten cc of blood are withdrawn from the vein in a syringe and mixed with 2 cc of a 14 per cent sodium oxalate solution. This has been shown to be an isotonic anticoagulant which does not change the volume of the cells‡. Heparin and hirudin may be used but they are expensive and often not available. This mixture of oxalated blood is then centrifuged in an ordinary small centrifuge tube for about fifteen minutes. The supernatant plasma is removed and discarded, using a capillary pipette with a rubber nipple attached. By carefully manipulating the same pipette the buffy coat containing the white blood cells can be sucked up and used to prepare smears on cover slips or slides. These are then allowed to dry and stained and examined in the usual way.

The method outlined above gives us a histologic picture of the white blood cell content of 10 cc of blood and will often clinch a diagnosis which could not be confirmed from films prepared from the usual single drop of blood.

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‡Haden R. L. Technique of Determination of Relative Mass, Individual Cell Volume and Volume Index of Erythrocytes of Man. J. LAB. & CLIN. MED. 15:736, 1930.

AN IMPROVED DISTILLING COLUMN*

BY CHARLES B. DEWITT, MEMPHIS, TENN.

THE distilling column shown in Fig. 1 was designed for close fractionation of small quantities of liquids having relatively high boiling points. It has been found satisfactory for separation of lower boiling liquids as well. It combines high efficiency with unusual strength and compactness and gives the operator control over the distillation to a degree that is unmatched by any other column the writer has used.

The device will be readily recognized as an adaptation of the West condenser* in combination with a Vigreux column. The inner tube is made from light or medium wall Pyrex tubing whose diameter is dependent on the length

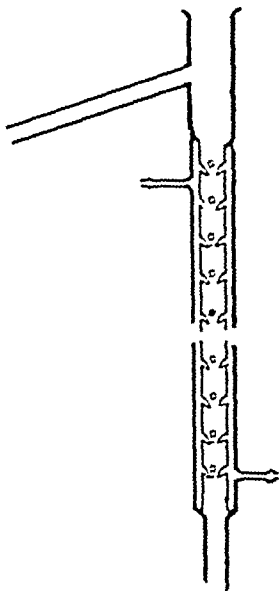


FIG. 1

of the column and the quantity of material to be used with it. An internal diameter of eight millimeters is sufficient for handling small quantities. The indentations are made by heating small spots in a sharp flame and pushing them in with a bent knitting needle. A deep file mark on the latter serves as a depth gauge. The indentations are made in groups of four to eight around the tube and the groups are spaced about three-fourths of an inch apart. This tube must be well annealed, particularly if the column is to be used for low pressure distillations.

*From the University of Tennessee.

Received for publication May 11 1931

†West, F. S. Industrial and Engineering Chemistry 20 No. 7 p. 737

The jacket is made of heavy wall tubing of a diameter that will allow a space of about one millimeter between it and the inner tube. Other construction details are clearly shown in the drawing. For the smaller sizes, the reducer shown at the lower end is unnecessary, as the jacket tube is small enough to go into the neck of a flask.

This column has no thin-walled bulbs or fragile siphon tubes that are easily broken in handling. The jacket, in addition to giving the column great strength, affords protection against air currents that often momentarily stop the output of distillate from other columns and cause exasperating fluctuations in temperature. To secure the necessary condensation, a stream of compressed air is led into the jacket through the upper side tube. This puts the heat transfer from the inner tube under control of the operator, and materially speeds up distillation. If a column of suitable size is used, it is seldom necessary to interrupt distillation to drain liquid from the column.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILGUFF, M.D., Abstract Editor

PNEUMOCOCCUS TYPING A Microscopic Method by the Use of Stained Organism Calder R. M. J. A. M. A. 97: 108, 1911

The organisms are prepared in suspension as for an ordinary microscopic typing and a drop is taken up by a capillary pipette and transferred to each of the four cover slips. Another capillary pipette is held for a moment in a Bunsen flame to seal its end and bend it into the form of a small hook. This hook is dipped into the gentian violet solution and the adhering dye is allowed to dry completely. The stain is worked into the drop of bacterial suspension on the cover slip and as the film of dye on the hook is very thin the amount mixed with the drop of suspension can be controlled accurately. The amount of dye to be worked into the drop to secure the best staining can be gauged easily after a little experience. If the dye as it comes off the hook spreads unevenly through the drop a second capillary hook, without stain, can be used to secure an even mixture. The organisms take up the dye selectively and appear as deeply stained bacteria in an unstained or faintly bluish medium.

After the drop has been stained equal volumes of the diluted serums are added by capillary tube and thoroughly mixed with the suspension by means of capillary hooks, care being taken, of course to use separate stirring rods for each of the various preparations so as not to mix the serums. In the ordinary typing four hanging drop preparations are set up, one for each of the three fixed types and one without serum for control. The cover slips are inverted over hollow ground slides, sealed and placed in the shaking machine where five minutes of shaking usually bring about sufficient agglutination to be read with the 4 mm objective.

ANEMIA Pernicious Relation of Achlorhydria to Moschcowitz, E. Arch. Int. Med. 48: 171, 1911

From a fairly comprehensive review of the subject the author concludes that

1 Achlorhydria is such a constant sign in pernicious anemia that probably no case is valid unless achlorhydria is present.

2 Achlorhydria is not the result of the disease but is primary. Evidence for this is shown in the fact that there is no diminution of hydrochloric acid in the progress of a case of pernicious anemia and that achlorhydria is present from the onset, that achlorhydria persists in the stage of remission and that it has been found frequently for years before pernicious anemia became manifest.

3 Achlorhydria occurs normally in a small percentage of persons. In order to determine this percentage, it is essential that mass studies be made with not only the fractional method of testing gastric secretion, but the tests with neutral red and histamine as well. The percentage of persons with achlorhydria apparently increases with each decade. It is extremely rare in childhood.

4 Achlorhydria is often present in certain families. In families in which a case of pernicious anemia has occurred, the incidence of achlorhydria is much higher than normal. As this incidence increases in each decade and as achlorhydria is exceptional in childhood achlorhydria itself is not transmitted but only the tendency thereto.

5 Pernicious anemia is frequently hereditary and familial. Whether pernicious anemia is always hereditary and whether it is a dominant or recessive character and transmissible according to the Mendelian law cannot be determined until such families are studied with

particular reference to achlorhydria and with accurate hematologic examinations being made for three generations at least

6 The available evidence is not convincing that *Bothriocephalus latus* causes pernicious anemia. There is ground for believing that such reported cases represent instances of true pernicious anemia that happen to be associated with infestation with *Bothriocephalus*.

7 There is a definite relation between achlorhydria and anemia, of the pernicious, secondary and chlorotic types. The blood of relatives of patients with pernicious anemia shows changes, which sometimes may be regarded as the earliest or preclinical evidence of the disease. For diagnostic purposes, it is important to recognize this preclinical phase.

8 The development of pernicious anemia after an acquired achlorhydria, for instance after complete gastric resection, has not definitely been proved.

9 Many of the so-called "severe anemias" of pregnancy (exclusive of those due to hemorrhage or sepsis) represent cases of true pernicious anemia in which the patients have become pregnant. Whether the pregnancy is the inciting factor in patients with a constitutional tendency remains to be proved.

10 The anemia of sprue is often associated with achlorhydria. It is not yet established how often achlorhydria is required or is a constitutional tendency in this disease.

11 Achlorhydria is the most tangible but not the only evidence of the constitutional background of pernicious anemia. In all probability pernicious anemia represents a combination of a deficiency disease and a lack of a gastric hormone.

TISSUE Modification of Mallory Heidenhain's Differential Staining Method and Adaptation to Formalin Fixed Material, Kernohan, J. W. *Am J Clin Path* 1: 90, 1931

The formalin fixed tissues are washed in running water or ammonia water for a short time. The latter procedure serves to prevent the accumulation of "formalin precipitate" on the stained preparation. The tissue is then placed for four days in Weigert's primary mordant for methyl sheaths (potassium bichromate 5 gm., chromium fluoride 2 gm., water 100 cc.), and for two days in Weigert's secondary mordant for methyl sheaths (acetate of copper 5 gm., chromium fluoride 2.5 gm., acetic acid, 36 per cent 5 cc., water 100 cc., formalin 10 cc.). It is then embedded in paraffin and stained in the usual way with Mallory's phosphotungstic acid hematoxylin or other staining methods requiring Zenker's fixation. This differentiates the neuroglia, fibroglia, etc., as satisfactorily and clearly as if the tissue had been fixed in Zenker's solution, and excellent results have been obtained after the tissue has been fixed several years in formalin.

VACCINE THERAPY Principles of, Kolmer, J. A. *Am J Clin Path* 1: 79, 1931

1 Vaccines have proved of more value in the prevention than in the treatment of disease.

2 Methods of preparation of stock and autogenous vaccines appear to have no important bearing upon their therapeutic value.

3 Successful vaccine therapy demands accurate bacteriologic diagnosis, especially the employment of proper and acceptable methods for securing the important organisms of infection for the preparation of autogenous vaccines.

4 Well prepared autogenous vaccines are to be preferred to stock vaccines in the treatment of disease.

5 Whenever possible vaccines should be composed of living organisms of reduced or modified virulence or so prepared as to approach this state as nearly as possible.

6 Bacteriologic toxins with or without modification should always be incorporated or used whenever possible in the preparation of vaccines.

7 The sterilization of vaccines with chemical agents or by filtration is to be preferred to sterilization by heat.

8 Prophylactic immunization is largely dependent upon passive antibody production whereas therapeutic immunization is probably due in part to both specific and non-specific effects.

9 The intracutaneous injection of vaccine may be of more prophylactic and therapeutic value than subcutaneous injection.

10 The route of administration and dosage of vaccine may vary in different groups of subjects and should be selected according to the indications and requirements of individual cases.

11 Vaccine therapy is sometimes of value in the treatment of cutaneous infection.

12 In the treatment of some chronic diseases vaccines have met with a measure of success and are worthy of further use especially by those possessing special skill and experience and with due regard for important technical details involving their preparation and administration.

TISSUE A New Clearing and Mounting Fluid for Small Insects Mulerji S. Ind. J. Med. Res. 19: 281, 1931

The following formula is presented:

Chloral hydrate	0.5 gm.
Distilled water	1 c.c.
Glycerine	1 c.c.
Lactic acid (extra pure)	2 c.c.
Glacial acetic acid	2.4 minims.
Formol	0.5 c.

The medium is easy to prepare and no filtering or heating is necessary since the fluids are perfectly miscible. When pure ingredients are used the resultant fluid has the advantage over lactophenol in that it is perfectly colorless and transparent. The ingredients should be mixed in the order given above and it is advisable to use a fresh medium each time.

Method of Clearing.—Some clearing medium is dropped with a pipette on the cell of an excavated slide and the entire object or its part as the case may be to be cleared is carefully dropped into the medium and the cell covered with a cover glass. Immediately the clearing of the parts begins to take place which should be watched through a binocular microscope. The medium may conveniently be used as permanent mountant with satisfactory results. A Nematode and a sandfly larva mounted in this medium more than a month back although sufficiently cleared have kept their original color.

URINE The Number of Formed Elements in Heart Disease Stewart H. J. and Moore N. S. J. Clin. Invest. 9: 409, 1930

The number of casts found in twelve hours is usually increased in patients suffering from cardiac disease, although the number may be normal. If the average numbers are considered, the greatest numbers were passed by those patients suffering from heart failure of the congestive type; the numbers were fewer after recovery and fewer still in those who had never suffered from this illness. Granular casts were frequently found.

The number of red blood cells in the urine of patients who had experienced cardiac decompensation was frequently greater than the highest normal value, but within the limits in those who had never suffered from heart failure. The average number of red blood cells found in those cases which had never experienced heart failure was twice as great as that in normal individuals; in those who were suffering from heart failure or had recovered from it, however, the average number was 10 to 15 times as great as in normal individuals.

The number of white blood cells was normal in the urine of those patients who had not suffered from heart failure but the average number was approximately twice the average observed in normal individuals. The number was usually within the normal range both during and after recovery from cardiac decompensation, the average number, however

was greater approximately 9 and 3 times respectively than that in normal individuals, the average being less in patients without heart failure than in those who had recently recovered from it and less than in those who were still suffering

LEPROSY Histamine Test in the Early Diagnosis of, Rodriguez, J and Plantilla, F C Phil J Sc 46 123, 1931

When a dilute solution of histamine is pricked into the normal skin, a reaction takes place in about twenty seconds, starting with the appearance of a circular, sharply defined, local reddening surrounding the prick, and measuring when fully developed from 3 to 4 mm in diameter. This is followed in another fifteen to thirty seconds by a flush or "flare" that appears on the surrounding skin. It is of the utmost importance to distinguish this flare from the local red reaction. The flare is dark red or scarlet contrasting with the brighter shade of the latter, it has diffused and often crenated borders that may extend from 2 to 3 cm from the center of the reaction. Soon after the appearance of the flare, a discrete wheal forms at the site of the prick, this is generally at its maximum development in from three to five minutes, it which time it measures from 3 to 4 mm in diameter and about 1 to 2 mm in height. The wheal usually occupies the area originally covered by the local red reaction, although in many cases the two do not coincide, the wheal being usually smaller than the localized red area.

The full reaction of the normal skin to histamine, consisting of the local redness or vasodilatation, the flare, and the edema or wheal has been called by Lewis the "triple response."

In most of the author's tests, they have used a 1 to 1,000 dilution of the phosphate in normal salt solution. With stronger solutions a larger flare is occasionally obtained, but the reactions are not as constant as with the 1 to 1,000 solution.

A small drop of the solution is carefully placed within the suspicious macule to be tested and another is dropped on normal skin at least 2.5 cm from the border of the lesion for control. With a sharp pin, a prick is made through the drop into the skin underneath, taking care to exert just sufficient pressure to drive the point through the epidermis without causing any bleeding. The histamine solution is wiped off immediately, and the pricks are closely observed under good natural light.

The test is said to be negative when the complete response is elicited and positive when the flare is absent.

There are some individuals on whom the normal reaction is diminished, in a few, the flare is so faint as to be practically absent. When the response is weak and the skin tested is on an extremity, the flare may be brought out to its maximum extent and intensity by previously congesting the extremity with the help of a broad rubber band or the pneumatic cuff of a blood pressure apparatus.

RESULT OF THE HISTAMINE TEST IN LEPROSY

In the pale macule. The flush is always absent in the depigmented macule of leprosy. When the histamine prick is made just outside the border, a flare develops on the normal skin but stops sharply at the border and does not extend into the macule. When the prick is made just inside the border, the flare is prevented from appearing even on the bordering normal skin.

A word of caution must be given at this point. The flare generally masks the local redness following the histamine test on the normal skin. When the flare is abolished as in a leprotic macule, the local redness becomes prominent and may be mistaken for the flare by the beginner. The area of local redness is sharply localized, circular in shape, bright red or pink in color, extending at the most 2 or 3 mm beyond the wheal, and tends to become cyanotic before fading. On the other hand, the flare is not definitely localized, the size is usually about 3 to 4 cm in diameter, irregular in shape, although it tends to be oblong with its long axis along the length of the member, and the color is dark red. On fading the flare becomes speckled, but the color remains the same from beginning to end.

The wheal in the muscle is usually of the same size as that on the normal skin. Sometimes the edema may be less, at other times the wheal develops faster in the muscle, reaching its full development in two minutes while the wheal on the control skin is at its height in three to five minutes. The ultimate size, however, is almost the same.

In the reddish muscle. When the redness of the lesion is marked, only the wheal may be elicited. But when the color is not so striking the local redness may be seen.

When hyperesthesia is present it is usually the case when the lesion is bacteriologically positive the flare is not constant. In a few muscles the flare is present in the majority of the cases it is absent. If there is accompanying infiltration or edema so marked that the skin looks tenfold turgid and bright red in color the wheal is apt to be slight or absent.

REVIEWS

Books for Review should be sent to Dr Warren T Vaughan, Professional Building,
Richmond, Va

Trauma, Disease, Compensation

THE title is at once suggestive, reminding the reader to what a great extent compensation features enter today into the treatment of injury and disease among the working classes. We find a comprehensive volume which on the whole, however is rather disappointing. The first chapter deals with the basis and scope of workmen's compensation and the discussion of the problem is very satisfactory, presenting as it does actual judgments rendered. From this point on, however, in the discussion of the various diseases which may or may not be come compensable, the information given does not seem to be quite definite enough. This appears to be due to the fact that with some exceptions, the references recorded are not from original sources or from legal decisions but from very general textbooks and systems of medicine. One would be unable on the witness stand to quote with any feeling of authority most of the statements made but would feel the need of first consulting the original authority and making a more detailed study of the specific problem.

Furthermore there are several subjects which come into everyday medico legal questions today which are scarcely touched upon. Such is the general problem of carbon monoxide poisoning.

The final chapter dealing with established ratings for permanent disability following accidents is helpful.

Unfortunately there are few authoritative volumes on this general subject and until more appear the present one will be of distinct aid. The reviewer hopes, however, that in his next edition the author will devote himself more to specific cases than to generalities.

Ideal Marriage Its Physiology and Technique†

THIS is the type of volume that can be reviewed in several ways. Many will declare that there is no justification for a treatise on the technique of coitus and that the volume should be suppressed. Others will hail it as a pioneer in the emancipation of current thought from the prudery of the past. The truth lies somewhere between.

The reviewer has felt that the test of the book is not in its reading but in its ability to solve the marital problems which its author assures us that it will help solve. For this reason we have delayed publication of the review for several months after the reading of the volume during which time we have used it in actual practice in a small selected series of situations in which it appeared that such a frank exposition would be desirable. From this study the reviewer has reached the following conclusion:

**Trauma, Disease, Compensation. A Handbook of Their Medico Legal Relations.* By A. J. Fraser, M.D., Chief Medical Officer, Workmen's Compensation Board, Winnipeg. Cloth. Pages 324. Philadelphia: F. A. Davis Company, 1930.

†*Ideal Marriage, Its Physiology and Technique.* By Th. H. Van de Velde, M.D., former Director of the Gynaecological Clinic at Haarlem. Translated by Stella Browne. Introduction by J. Johnston Abraham, C.B.J., D.S.O., M.A., M.D. Cloth. Pages 323. New York: Cowell-Friede, 1928.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

While the flavor of the volume is distinctly continental and the author gives to the art of intercourse a glory and importance which is scarcely bestowed upon it in America there is at the same time no doubt that many of the mistakes especially among women are based upon sex and are often due to failure of sexual stimulation. There is no doubt that much of this is due to ignorance on the part of the mate. The reviewer has been able to clear up such situation entirely by showing both parties to study the book.

It is a volume which in the hands of the light minded could do much harm. It is one which should be read by all physicians who are seriously endeavoring to better the lot of their patients. It is one which is unhesitatingly to be recommended to the whole physician feels are in need of the information contained therein and who are capable of intelligently interpreting the information.

Legal Medicine and Toxicology

WHILE it is true that in medical specialization there has developed a group of specialties related to expert testimony such as the toxicologist and the chemist it is equally true that the ordinary doctor finds him self called into court with increasing frequency. Sometimes he is called as an ordinary witness, often as an expert. It becomes increasingly desirable that the doctor have available for his instruction reference volumes on the doctor as an expert in court. Most of the problems which he will be called upon to face are treated in Webster's textbook.

Thus, in the first section on legal medicine subjects such as the following provide the physician with a wealth of information on the basis of which he is better equipped to conduct himself properly on the witness stand. "Legal Procedure, The Coroner's Inquest, The Right to Practice Medicine, Contractual Relations Between Physician and Patient, Privileged Communications, Dying Declarations, The Making of Wills, Obligations of the Physician to the State, Death Certificates and Birth Certificates, The Prescribing of Narcotics and Alcohols, Malpractice, Criminal Malpractice, Identification of the Living and the Dead, Death in Its Medical and Legal Relations, Impotence and Sterility, Fugue, Legitimacy, Paternity, Abortion, and Mental Disorders in Their Medical and Legal Relations." Subjects such as these come into the problems of every physician at one time or another.

The second section devoted to Toxicology represents a more highly specialized field one in which the general practitioner need never qualify himself but at the same time one in which the clinical pathologist should be well grounded.

Doctor Webster's position as professor in medical jurisprudence in Rush and his former association with Peterson and Humes in their volume on Legal Medicine and Toxicology insure to the reader an authoritative exposition of the subject.

*Legal Medicine and Toxicology. By Ralph W. Webster, M.D., Ph.D., Clinical Professor of Medicine (Medical Jurisprudence) in Rush Medical College, University of Chicago, Professorial Lecturer in Medical Jurisprudence and Toxicology in the University of Chicago, Toxicologist to the Coroner's Office, Cook County, Illinois, Attending Chemist, Presbyterian Hospital, Chicago, Director of Chicago Laboratory, Clinical and Analytical. Illustrated. Pages 562. Cloth. Philadelphia and London W. B. Saunders Company, 1920.

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EDITORIAL

The Effect of Carbon Monoxide Poisoning on the Heart

CARBON monoxide exerts its poisonous action by combining with the hemoglobin of the blood thereby depriving the blood of oxygen. The affinity of carbon monoxide for hemoglobin has been estimated as being about 250 times the affinity of oxygen for hemoglobin. Therefore, when once carbon monoxide has become attached to hemoglobin, oxygen is crowded out as it were, and, being deprived of its method of transportation through the blood to the tissues, it can no longer reach the latter. As a consequence the cells of the organs and tissues of the body are virtually asphyxiated dying for lack of oxygen. The most striking anatomic change resultant on this consists in minute hemorrhages, blood passing out through the damaged blood vessel walls into the tissues. This may occur in any tissue of the body, but since the nerve cells of the central nervous system are the most sensitive to oxygen deprivation, the most characteristic and constant findings in carbon monoxide poisoning are in the brain. At autopsy small hemorrhages or extravasations are found scattered through the brain substance. If the victim lives long enough these hemorrhagic areas soften undergoing cystic degeneration.

While it is stated the findings in the central nervous system are the most characteristic and the most constant, similar changes may occur in other organs of the body. It was not until 1919 that any special study appears to have been made of the effects of carbon monoxide poisoning on the heart.

In this year Zondek of the University of Berlin made careful studies of three cases of illuminating gas poisoning. All three recovered. In addition to the usual clinical studies careful records were kept of the pulse and the blood pressure and X-rays were made repeatedly to determine the size of the heart. As a result of his studies Zondek concluded that even in patients who recover from the effects of gas poisoning there is definite evidence of damage to the heart. He described a constant and diagnostic complex consisting of (1) a pronounced fall of blood pressure which usually lasts for about a week, (2) rapid pulse at the beginning followed usually on the third or fourth day of convalescence by a pronounced slowing of the pulse to distinctly below the normal rate. This also lasts several days. (3) There often also develops irregularity of the heart usually consisting of extrasystoles or a pronounced respiratory arrhythmia. His x-ray studies demonstrated (4) a definite dilatation of the heart following gas poisoning usually beginning very shortly after the poisoning with a recovery from the dilatation after three or four days. The degree of dilatation appeared to vary depending upon the previous health of the heart. In one case that of a thirty-seven old man the dilatation did not appear until the third day and in this case at the end of two weeks the patient was still suffering from symptoms due to the "cardiac dilatation." Zondek speaks of this type of reaction as delayed dilatation.

In the same year H. Lachmann of the University of Munich confirmed the work of Zondek in a report in which he stated that he also had observed several cases with similar heart changes. He also reported the autopsy findings in a case of illuminating gas poisoning in which he found multiple hemorrhages in the brain substance and an interstitial and parenchymatous myocarditis which he felt was due to the effects of the gas poisoning. This investigator had failed to demonstrate changes in the heart muscle in experimental animals following carbon monoxide poisoning, but in this connection he remarked "nevertheless the case which I have just described must be considered proof that carbon monoxide poisoning exerts a direct toxic effect on the heart muscle which in severe cases manifests itself as an interstitial and parenchymatous myocarditis." Concerning failure to find these heart muscle changes more often at autopsy he states that with the methods of microscopic examination that are available the myocardial changes are of such nature that they can easily be overlooked and indeed can only be found after prolonged study with serial sections.

In 1920 G. Herzog reported his study of the pathology of illuminating gas poisoning before the Medical Society of Leipzig. He found as regards the heart, that eight out of ten autopsies of persons dead from gas poisoning showed evidence of damage to the heart muscle. He described the microscopic findings as being chiefly hemorrhagic necrosis and leucocytic infiltration. In one woman of thirty years who died three days after the poisoning there was a very pro-

nounced dilatation of the left side of the heart. One child who died twenty three days after gas poisoning showed marked changes in the heart muscle.

In 1925 Gurich of the Pathologic Institute of the Hamburg-Eppendorf General Hospital again confirmed the work of these other investigators, adding additional cases of his own. In three of four fatal cases he found the characteristic heart lesions. He remarked on the occurrence of the minute hemorrhages in the heart muscle similar to those found in the brain. He further remarked on the great variation of the reactions and findings in individual cases. One person may manifest serious damage while another shows little or none of a permanent nature. For example, two brothers who died at the same time from gas poisoning. One showed the characteristic heart changes while the other failed to do so. This author believes from his studies that the gas acts as a direct poison to the heart muscle cells.

Concerning the ultimate changes in the hearts of persons who recover from gas poisoning we can of course have no information but Gurich believes from his studies that there probably remains some permanent damage with scar tissue formation in the heart.

In 1930 Martin Israelski and Ernst Lucas, working in Zondek's Clinic described two additional cases of illuminating gas poisoning both of which eventually recovered, in both of which x-ray examination showed evidence of hemorrhage into the lungs and in one of which there was a dilatation of the heart similar to that previously described by Zondek.

Howard W. Haggard in 1921, studying experimental carbon monoxide poisoning in animals concluded that there was no direct action of this poison on the nerves controlling the heart. It will be noted in this connection that the work of the German investigators above described indicated that the poisonous effect of gas on the heart was not on the nerves controlling the heartbeat but on the heart muscle itself.

In comparing the poisonous effect of pure carbon monoxide gas with that of illuminating gas Haggard found that the accessory toxic substances present in illuminating gas in addition to carbon monoxide caused more rapid asphyxiation than pure carbon monoxide, due to the fact that they stimulated the respiration, thereby hastening the development of respiratory fatigue and failure but under the conditions of the experiment, he found no evidence that these accessory substances were in and of themselves directly or rapidly toxic.

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CLINICAL AND EXPERIMENTAL

THE DECOMPENSATED HYPERTENSIVE HEART*

BY JAMES R. LISA, M.D., NEW YORK, N. Y.

DURING the course of studies on hypertension certain changes in the heart were found which seem to have some relationship to the decompensation which sometimes occurs.

This report is based on ten cases. They all had one feature in common, congestive type of cardiac failure with dyspnea on exertion, cyanosis and edema. The blood pressures ranged from 160 to 255.

The hypertrophy was marked in all, the weights ranging from 450 gm. to 1110 gm. With the exception of the presence of many small scars the myocardium presented no marked gross changes. Infarction was absent in all. Its consistence was more or less diminished and its color slightly mottled yellow-brown red. Microscopically very numerous necrotic foci were found, most frequent in the left ventricle and the interventricular wall though present to a much lesser degree in the other chambers.

The youngest lesion involves only an individual or a very few fibers and consists of a granular degeneration of the cytoplasm, sometimes with, many times without, a cellular infiltration. When present it is usually polynuclear but rapidly becomes mixed cell in type. The cytoplasm rapidly disintegrates and apparently liquefies, when the nuclear remains and the infiltration of cells are more prominent. The edges of the adjacent fibers have cross striations consisting of a row of coarse granules. The lesion sometimes seems to be of more rapid evolution and leaves only a network of capillaries with disintegrating muscle fibers and very slight cellular reaction. Occasionally the cell reaction is of a sharply polynuclear character. More frequently it consists

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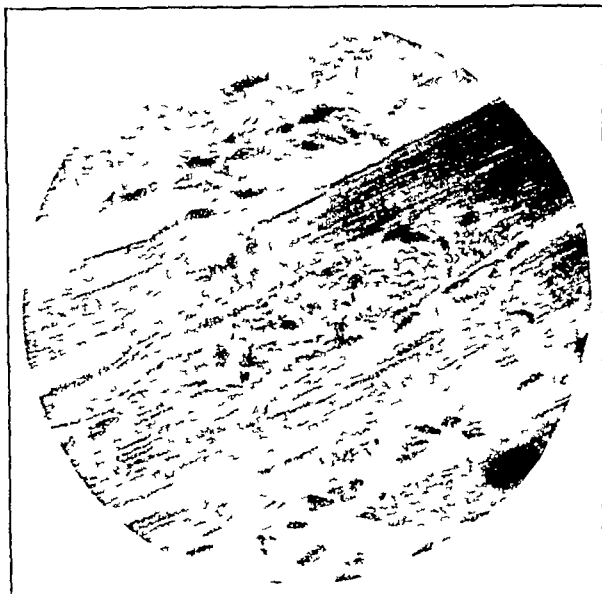


Fig 1—Early acute millary necrosis showing granular degeneration and slight infiltration. In the upper portion of the field is the edge of neighboring lesion showing the fibrillary structure and early connective tissue reaction

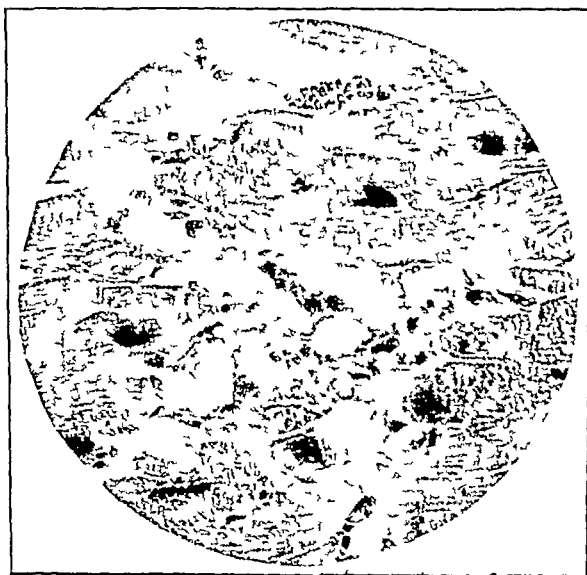


Fig 2—A slightly more advanced lesion with almost complete disappearance of the cytoplasm and cellular reaction of lymphocytes monocytes and a very few polynuclears. The neighboring fibers have the earlier granular appearance and loss of striations

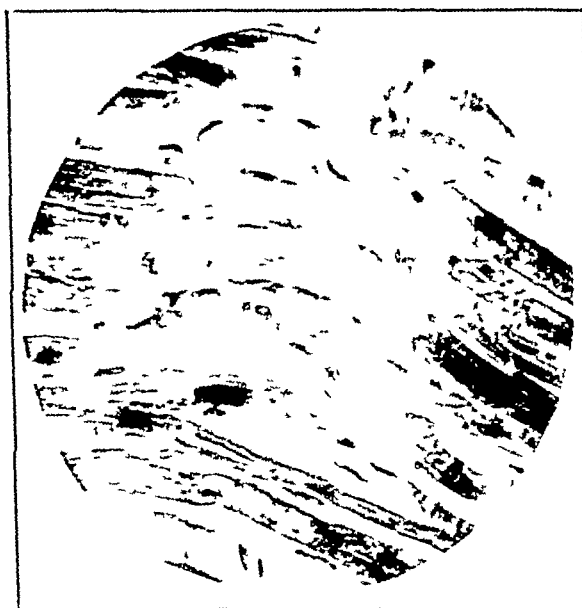


Fig 3—A more fulminant type of lesion with extremely acute degeneration of the muscle fibers leaving the capillary network and with very mild infiltration along the edge.

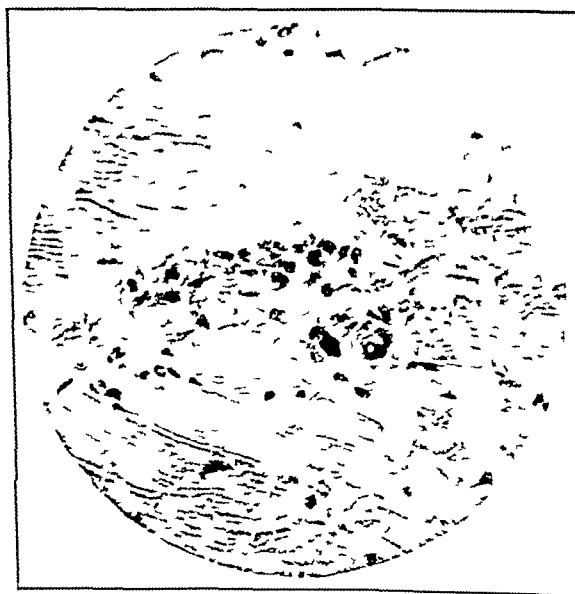


Fig 4—A slightly later stage than Fig 3 with a marked cellular reaction chiefly monocyte.



Fig 5 —A later phase with the predominant infiltration lymphocytic in character and with early connective tissue reaction

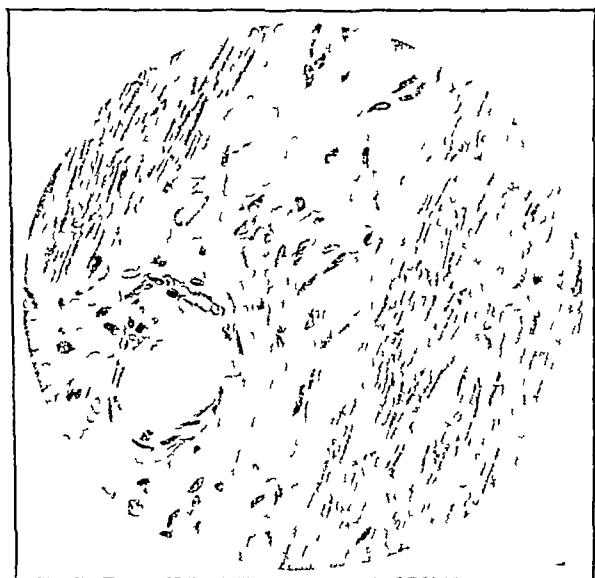


Fig 6 —A milium scar with young connective tissue and almost complete disappearance of lymphocytes

of polymorphous monocytes and lymphocytes in varying proportion. As the connective tissue begins to proliferate, the lymphocytes tend to predominate and finally completely disappear leaving only a military scar.

Associated with these necroses the myocardium presents a marked fragmentation and areas of fine wavy fibrillae. These resemble very closely some of the lesions found in isolated acute myocarditis.

Three of the cases were associated with syphilis. Spirochetes however could not be demonstrated in any of the hearts from these cases. Syphilis was most likely simply an associated disease unrelated to the hypertension. All the cases were examined for the presence of bacteria with negative results.

CONCLUSIONS

- 1 Microscopic lesions of the heart in a series of ten cases of hypertension with congestive cardiac failure are presented
- 2 They consist of military necroses with cellular reaction
- 3 The histogenesis is traced
- 4 They apparently have a close relationship to the clinical syndrome and offer a pathologic basis for it

Thanks are due to Miss I. B. Miller for the microphotographs.

RELATION OF JAUNDICE TO THE OUTCOME IN LOBAR PNEUMONIA INDICATIONS FOR THE TRIAL OF BILIRUBIN THERAPY*

By NORMAN W. ELTON, M.D., D.N.B., BOSTON, MASS.

IN SEPTEMBER 1928, a study was undertaken to determine whether or not jaundice was constantly associated with lobar pneumonia,¹ and if so, to analyze its changes in character and intensity in relation to the progress of the disease during its acute course. Such a study was considered advisable because of the possibilities suggested by the presence of jaundice in a disease caused by a bile soluble organism.^{2,3} The method consisted of the daily determination of three tests with blood serum, the icterus index,⁴ the aqueous (direct) van den Bergh reaction,⁵ and the quantitative serum bilirubin estimation.^{3,1} The study was based entirely on qualitative and quantitative findings in terms of these three tests, and the trend of the icterus, shown in the daily changes in each test, was correlated as closely as possible with the events of the disease. Hence, it may be described as a study of icterus kinetics. At present 224 cases have been observed in this manner from four different hospitals (Highland Park General Hospital, Michigan, Detroit Receiving Hospital, Michigan, St. Mary's Hospital, Detroit, Michigan, and the Boston City Hospital, Massachusetts). Icterus, manifested in one or all of the three tests, was found to be practically universally present in lobar pneumonia, although it was often encountered in a receding phase when the patient was first seen.

The work had not progressed far before it became evident that it was not a simple matter, but had many different aspects which required detailed consideration. No short space of time was consumed in becoming familiar with the technique and interpretation of the three tests and it was found necessary, in order to understand the icterus of lobar pneumonia, to undertake an extensive study of jaundice as it runs its course in other clinical entities. The results of this extension of the scope of the investigation are published separately.⁶

During the study of the first series of pneumonias many confusing factors were involved, particularly the outbreak of an influenza epidemic in 1928-1929, during which many cases were quite atypical. Confusion arose from an attempt to classify cases on the basis of the temperature charts, and it was later found that the key to proper classification was the recognition of another factor related conjointly with icterus to the outcome, fluid pleural exudate. The study of these exudates became fully as important as the study of the icterus itself, for when they were present not only did the icterus tend to recede, but the mortality rate was greatly reduced.

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To present the findings as clearly as possible it has been necessary to classify cases on the basis of the presence or absence of pleural fluid in relation to the maximum icterus index attained during the acute course. Until the recent series at the Boston City Hospital were studied by routine chest tapping many cases could not be placed in either of these groups because of incomplete data. Although in most of such doubtful cases recovery with effusion was indicated by the dropping of the icterus index to normal during the acute course. It was evident that whenever pleural fluid was diagnosed by x-ray, chest tap, or careful physical examination, the drop of the icterus index signaled its presence either as or shortly after the fluid developed, but it remained to be demonstrated that whenever this drop occurred fluid should be expected. For this reason it was essential to obtain conclusive data by routine chest tapping in an adequate number of well studied cases.

1 *Highland Park General Hospital ("Orientation Series") Table I*—This series comprises 70 cases in white adults studied during the year 1928-1929. Influenzal cases are included, accounting for many of the fatalities in the normal zone of the icterus index. The clinical data on these cases have already been published. They are here reclassified on the basis of the presence or absence of pleural fluid. Certain observations applicable to the white race may be made from a study of this chart which are consistently upheld by those that follow. (1) In the absence of pleural fluid the mortality is 100 per cent unless the icterus index exceeds 16.6. (2) In the icterus index zone 18.7-30 there is no mortality with or without fluid. (3) In the presence of fluid patients entering the 11-15 zone of the icterus index invariably recover.

TABLE I
HIGHLAND PARK GENERAL HOSPITAL
ORIENTATION SERIES
WHITE RACE

MAXIMUM ICTERIC INDEX ATTAINED DURING ACUTE COURSE	TOTAL CASES	NO FLUID		FLUID EFFECT		SUSPECTED FLUID		TOTAL DEATHS
		NO	CASES DEATHS	NO	CASES DEATHS	NO	CASES DEATHS	
6	27	1	1	17	4	0	3	8
7-10	19	8	8	5	1	6	1	10
11-15	15	4	4	5	0	6	0	4
16-6	5	3	3	0	0	0	0	3
18-7-30	6	4	0	2	0	0	0	0
Totals	70	20	16	29	5	21	4	25

2 *Detroit Receiving Hospital White Race (Including Cases Observed at St. Mary's Hospital, Detroit, Michigan) Table II*—This series comprises 74 white adults studied during the year 1929-1930. Positive diagnosis of fluid was made in 12 by x-ray, in 6 by physical examination, in 9 by chest-tap, and in 1 by autopsy. There were 10 autopsies in cases when death occurred without fluid. The greater number were typed and many had serial blood cultures. In two cases the icterus index exceeded 30, one patient dying with a type II septicemia at 37.5, the other recovering by true crisis at 100. Several of these cases were obtained only by autopsy and the icterus

determined in the postmortem blood, which partially accounts for the relative increase in the number of deaths with dry pleural cavities. It was impossible to study many patients who may have recovered with fluid, since only those in Detroit Receiving Hospital were closely observed.

TABLE II
DETROIT RECEIVING HOSPITAL SERIES
WHITE RACE

MAXIMUM ICTERUS INDEX ATTAINED DURING ACUTE COURSE	TOTAL CASES	NO FLUID			FLUID PRESENT			SUSPECTED FLUID			TOTAL DEATHS
		NO	CASES	DEATHS	NO	CASES	DEATHS	NO	CASES	DEATHS	
6	11	0		0	8		1	3		0	1
7 10	30	9		9	12		4	9		0	13
11 15	17	9		9	7		0	1		0	9
16 6	5	5		5	0		0	0		0	5
18 7 30	9	7		0	1		0	1		0	0
37 5	1	1		1	0		0	0		0	1
100	1	1		0	0		0	0		0	0
Totals	74	32		24	28		5	14		0	29

3 *Detroit Receiving Hospital Black Race Table III*—From a study of but 46 cases in negroes, it is at once evident that the relationship of icterus to the outcome is strikingly different in this race. Fluid was diagnosed in 15 cases by x-ray, in 6 by physical examination, in 4 by chest-tap, and in 1 by autopsy. There were 6 autopsies in patients dying without fluid. From the data of this table the following observations may be made: (1) The icterus index 16.6 bears no significance. (2) In the absence of pleural fluid the mortality is 100 per cent unless the icterus index exceeds 100. (3) In the presence of fluid, although the mortality rate is greatly reduced, no zone of assured recovery can reasonably be indicated in only a single series. The similarity between the curves of the icterus index in negroes with those of patients apparently white who exceed 30, is striking, for in the absence of fluid the end point for recovery appears to be the same (icterus index 100).

TABLE III
DETROIT RECEIVING HOSPITAL SERIES
BLACK RACE

MAXIMUM ICTERUS INDEX ATTAINED DURING ACUTE COURSE	TOTAL CASES	NO FLUID			FLUID PRESENT			SUSPECTED FLUID			TOTAL DEATHS
		NO	CASES	DEATHS	NO	CASES	DEATHS	NO	CASES	DEATHS	
6	12	1		1	8		3	3		0	4
7 10	9	1		1	7		2	1		0	3
11 16 6	11	2		2	7		0	2		0	2
18 7 30	6	3		3	2		0	1		0	3
37 5	2	2		2	0		0	0		0	2
50	1	1		1	0		0	0		0	1
75	1	0		0	1		1	0		0	1
84	1	1		1	0		0	0		0	1
85 7	1	0		0	1		0	0		0	0
120	1	1		0	0		0	0		0	0
222	1	1		0	0		0	0		0	0
Totals	46	13		11	26		6	7		0	17

TABLE IV
BOSTON CITY HOSPITAL SERIES,
FIFTH MEDICAL SERVICE
WHITE RACE

MAXIMUM ICTERUS INDEX ATTAINED DURING ACUTE COURSE	TOTAL CASES	NO FLUID		FLUID EFFUSION		TOTAL DEATHS
		NO CASES	DEATHS	NO CASES	DEATHS	
6	7	1	1	6	0	1
7-10	11	3	3	8	2	5
11-15	8	2	2	6	0	2
16-6	2	1	1	1	1	2
18.7-30	1	2	0	2	0	0
43	1	1	1	0	0	1
100	1	1	0	0	0	0
Totals	34	11	8	27	3	11

TABLE V
CLINICAL SUMMARY

SERIES AND YEAR	INCIDENCE OF EFFUSION		EFFUSION TO MORTALITY		
	PROVED	PROVED AND SUSPECTED	MORTALITY WITHOUT FLUID	MORTALITY WITH FLUID	
				PROVED	PROVED AND SUSPECTED
Highland Park General Hospital, Michigan, 1928-1929	41.4%	71.5%	50.0%	17.2%	18.0%
Detroit Receiving Hospital, Michigan 1929-1930	37.5%	56.8%	75.0%	17.8%	11.9%
Detroit Receiving Hospital, Black Race 1929-1930	56.5%	72.0%	84.6%	23.0%	18.2%
Boston City Hospital Mas- sachusetts, 1930-1931	67.6%	—	72.7%	13.0%	—
Total Series, Percentages for White Race	47.0%	64.6%	76.2%	16.3%	14.8%

4 *Boston City Hospital, Fifth Medical Service White Race Table IV*—This group of 34 cases studied during the year 1930-1931 is presented in detail on the chart of the clinical protocol as well as on the general classification table. There were none in which doubt existed as to the presence or absence of fluid. From a study of the findings in the white race the following observations can be made: (1) In the absence of fluid the mortality is 100 per cent in the icterus index zones "normal" to 16.6, and above 30 to an undetermined point below 100. There were no deaths in the zone 18.7-30, or, when the icterus index exceeded 30, if it attained 100. (2) In the presence of pleural fluid there was no mortality if the icterus index exceeded 10 but did not attain 16.6. (3) *Pneumococcus septicemia* tended to subside spontaneously in the presence of effusion (noted also in the Receiving Hospital series when no specific antibodies were given). (4) *Pneumococcus septicemia* was often demonstrable with icterus indices at or below 16.6, and occasionally above 30, but has not yet been encountered in cases entering and not exceeding the zone 18.7-30, except in the negro race. (5) Prognoses could not be made from the visible manifestations of icterus, but icterus could be seen in a large number of cases if the sclerae were carefully examined daily. (6) Profuse perspiration frequently accompanied the development of fluid. (7)

TABLE VI
CASE PROTOCOL BOSTON CITY HOSPITAL SERIES 1930 1931

SERIES NO	SEX AND AGE	TYPE	DURATION OF PHYSICAL SIGNS OF FLUID	DAY OF TAP	DESCRIPTION OF FLUID	CHEST TAPS				KINETICS OF SPERMICITRUS	CLINICAL DATA	BACTERIOLOGIC AND PATHOLOGIC DATA
						ICTERUS IN FLUID			FLUID CULTURE			
						ICTERUS INDEX	DIRECT REACTION	BILI RUBIN				
1	♂ 42	XVIII	None	4	Dry					4th day Index 6 Direct reaction del 4 min Bilirubin 0.5 5th and 7th days Index 5.5 Direct reaction negative Bilirubin Below 0.2	Died 4th day Under influence of Acronal (addict) Entire left lung consolidated Temp 104 pulse 120 160 resp 60 No sputum Consolidation R middle lobe on 4th day (x ray) interlobar fluid on 9th day (fluoroscopic) T P R septic to 10th day	Type 18 obtained by lung puncture WBC 24 000 polys 91% Blood culture negative 3d day Blood culture negative 6th day WBC 22 000 polys 80% 5th day
2	♂ 38	I	None									
3	♂ 36	I	2d to 6th day	4	100 cc Amber Turbid		10	Neg	0.6	N G	Chill rusty sputum for 24 hr while on ward recovering from acute rheumatic fever Right br c Temp fell to normal from 103 following tap Rusty sputum first 4 days Private patient of Dr John A Foley	Cell studies typical of pneumonic effusion
4	♀ 21	VIII	6th to 12th day							9th day Index 5 Direct reaction positive Bilirubin 0.33		Type 8 from sputum
5	♂ 47	III	Constant	14	60 cc Amber Turbid					N G	Admitted because of persistent dyspnea following chill 11 days before No rusty sputum Herpes on lips No history or signs of tuberculous Right chest T P R septic through out Relieved in another hospital	Type III from sputum Sputum consistently negative for tubercle bacilli Guinea pig inoculation with fluid negative Cell studies typical of pneumonic effusion with no evidence of empyema WBC 20 500 21 980 19 900 14 550 polys 90% 80% 80% 67%
				16	60 cc Amber Turbid					N G		
				19	60 cc Amber Turbid					N G		
				27	60 cc Yellow Clear					N G		
				39	60 cc Yellow Clear		3	Neg	0.22	Guinea pig neg		
6	♂ 18	I	3d to 5th day	4	10 cc Green Turbid		5	Neg	0.68	N G	Temp fell by crisis on 5th day from 103 before serum could be given X ray negative on 4th day	Blood cultures negative 1st and 4th days WBC 26 000 polys 95%
				5	10 cc Yellow Turbid					N G		
				6	8 cc Yellow Turbid					N G	Treated with Felton's specific antibodies 6th and 7th days Temp fell by crisis on 8th day	Blood cultures negative 6th to 8th days WBC 25 000 polys 92%
7	♂ 21	I	6th to 10th day	8	2 cc Yellow Clear					N G		

Q. T-CONT'D

[illegible]

TABLE VI—CONT'D

SERIES NO	SEX AND AGE	TYPE	DURATION OF PHYSICAL SIGN OF FLUID	GUEST TAPS					KINETICS OF SERUM ICTERUS	CLINICAL DATA	BACTERIOLOGIC AND PATHOLOGIC DATA
				DAY OF TAP	DESCRIPTION OF FLUID	C-TEST INDEX	D RECT RE ACTION	FLUID CUL TURE			
15	♂ 22	Group IV	6th to 13th day	9	30 cc Brown Turbid	6	Del t Min	0.48 N G	From 6th day Index 6 10 6 5 4 3 35 35 Direct reac del 4 pos to 11th day Bilirubin 0.4 0.5 0.56 0.44 0.2 0.2 Z 2	Treated with types I and II antibodies on 6th day terminated 10th day lower lobe	
16	♀ 43	XIV	9th to 15th day						From 9th day Index 6.4 7.5 6 Direct reac pos pos neg Bilirubin 0.46 0.41 0.25	Left lower lobe Temp down by lysis 10th day WBC 12,800 on 12th day	
17	♂ 19	I	9th to 11th day	9	1 cc Yellow Turbid			No or gan isms	From 2d day Index 6 5 7.5 10 4.3 11th day 4.3 Direct reac del 2 pos to 6th day neg Bilirubin 0.36 0.38 0.5 0.2 11th day Z 2	Influenza on et 2d attack in 6 mo Specific antibody therapy Right upper lobe Fluid obtained from base	Blood cultures negative 2d to 5th days WBC 26,300 polys 87% Kahn and Wassermann positive
18	♂ 27	I	6th to 12th day	5	0.5 cc Amber Turbid			N G	From 4th day Index 7.5 7.5 5.3 4.3 5 6 3 Direct reaction del 2 1/2 pos, pos del 5 neg Bilirubin 0.5 0.2 0.2 0.33 0.23 Z 2	Specific antibody therapy 5th day Crisis on 6th day Delirium tremens Right lower lobe	Blood cultures negative 4th and 5th days WBC 22,000, polys 92% 4th day WBC 11,600 polys 84% 9th day
				9	20 cc Amber Turbid			N G			
19	♂ 45	I	None						From 4th day Index 13.6 11.6 15 11.6 10 15 Direct reaction positive throughout Bilirubin 0.91 0.6 1.0 0.62 0.3, 0.5	Died 9th day Non-specific (antimeningococcal) serum therapy 4th day Right mid lobe only up to 6th day Seemed to be doing well until 7th day Sclerotic icteric	Blood cultures negative 4th day positive 6th day Autopsy—gray consolidation of all lobes except part of apices Drs pleural cavity

TABLE VI—Continued

SERIES NO.	SEX AND AGE	TYPE	DURATION OF PHYSICAL SIGNS OF FLUID	CHIEF TAPS					ANALYTICS OF SERUM ICTERUS	CLINICAL DATA	BACTERIOLOGIC AND PATHOLOGIC DATA	
				DAY OF TAP	DESCRIPTION OF FLUID	ICTERUS IN FLUID						FLUID CULTURE
						ICTERUS INDEX	DIRECT REACTION	BILIRUBIN				
26	♂ 42	I	10th to 18th day	10	Dry					From 5th day Index 5 10 8 10 12 5 6 8 7 5 6 6 Direct reaction positive to 14th day, neg. nec. Bilirubin 0.32 0.9 0.77 1.0 0.52 0.5 0.1 0.25 2.2	Treated with specific antibodies on 10th and 11th days. Septic by 11 terminated 20th day. Sclerotic icterus. Left lower lobe.	Blood cultures positive 8th to 10th days, negative 7th and after 10th days. WBC 6,800—11,000 8th 9th day. Kahn and Wakserman positive.
				12	25 cc Amber Turbid	12.5	Pt	0.7	N C			
				15	50 cc Amber Clear				N G			
27	♂ 47	III	None	7	Dry				From 7th day Index 8 12 11 8 15 16.6 Direct reaction positive throughout Bilirubin 0.5 1.0 0.9 1.0 1.05	Died 11th day. Right lower lobe. Temp 99.6 at death following dose of types I and II antibodies. Sclerotic icterus.	Blood cultures positive daily—150 col per c.c. on 10th day. Type III from postmortem lung puncture. WBC 8,800 to 9,400 polys 92%.	
				10	Dry				N C			
28	♂ 47	II	7th to 10th day	7	2 cc Yellow Turbid				6th day 10th day Index 10.7 16.6 Direct reaction neg. nec. Bilirubin 0.5 1.54	Died 10th day at death entire right lung consolidated. Sclerotic icterus.	Blood culture positive 7th day. WBC 14,500 polys 88% 7th day. Autopsy—ery consolidation of all right lung and left upper lobe. Left lower red. R pleural cavity 250 cc fluid left 150 cc.	
				8	Dry							
29	♂ 45	I	None	8	Dry				From 5th day Index 15 30 30 16.6 8 9 3 7 6.2 6 Direct reaction positive to 12th day, neg. nec. Bilirubin 0.57 1.4 1.6 0.84 0.25 0.4 0.23 0.25	Right middle lobe only (x-ray). No signs in chest after 8th day. Rusty sputum to 6th day. Temp fell to normal 7th day. Sclerotic icterus.	WBC 10,000 5th day. Spectroscopic examination of serum on 6th 7th days showed only obliteration of the blue violet and bands of oxyhemoglobin.	
				9	2 cc Amber Turbid							
30	♂ 38	I and VIII	6th to 8th day	7	2 cc Amber Turbid				From 6th day Index 7 5 6 6.9 12 5 10 8 11 5 8 5 6 6 5 Direct reaction negative to 14th day, del. 14 20th day del. 2 neg. Bilirubin 0.58 0.25 2.2 1.0 1.0 0.6 0.5 0.4 0.3 2.2	Nonspecific protein therapy 6th and 7th days. During recovery from type I infection became irritable and toxic on 12th day with type 8 R lower lobe. True crisis on 14th day. Sclerotic icterus.	Blood cultures negative 6th 7th and 13th days. WBC 11,000 on 7th and 23d days.	
				9	Dry							

Spontaneous sudden termination of the fever, followed by uneventful recovery, was not uncommonly observed in the first few days of illness without specific treatment when fluid was present (6) All but type I or II patients treated early with Felton's concentrated antibodies recovered with pleural fluid In the fatal case fluid was also present

GENERAL CHARACTERISTICS OF THE ICTERUS OF LOBAR PNEUMONIA

The data given in the clinical protocol of the Boston City Hospital series shows the fluctuations of the pneumonic icterus in specific cases This icterus is not to be regarded as specific for pneumococcus pneumonias for it has also been observed in three cases due to Friedlander's bacillus, one dying at the index 25, another at 60, another with effusion Since Friedlander's bacillus is not known to be bile-soluble it is obvious that the icterus cannot be interpreted in the same way as for the pneumococcus Since the hemolytic streptococcus and the staphylococcus do not, per se, produce pathologically the picture of lobar pneumonia, although they may produce clinical signs simulating lobar pneumonia and they also are not bile soluble, icterus again cannot be regarded in the same light as when the pneumococcus is the invader

The zoning of the pneumonic icterus on the basis of the icterus index rather than on the quantitative bilirubin, alone makes possible a seemingly consistent classification The van den Bergh reaction usually becomes direct positive at some time during the acute course of any lobar pneumonia, but does not afford a method of classification In all cases terminating fatally, with the exception of two dying during the first four days of illness, the direct positive reaction has been present on the day of death Following recovery without fluid (true crisis) it has persisted direct positive for several days Accompanying recovery with fluid it gradually weakens, then frequently suddenly disappears completely, becoming negative in ten minutes without going through the development of and progressive lengthening of the delay period of the delayed reaction Delayed resolution with fluid appears to be measured by the duration of this direct positive reaction even though the icterus index and quantitative bilirubin have already dropped to normal

The quantitative bilirubin, per se, cannot be used as a basis for classification, but exhibits certain striking characteristics in its relation to the icterus index in the white race, particularly when the reaction curves are associated with the true crisis phenomenon (recovery without fluid) This mechanism accompanying true crisis has proved difficult to isolate for satisfactory study because it seems quite rare and the changes may take place so rapidly that they may elude the twenty-four hour determinations However in some slower cases it has been evident, and it corresponds closely to a similar reaction occurring during the ascent of icterus often encountered in familial jaundice, pernicious anemia, the newborn, and occasionally following a traumatic interstitial blood extravasation

The characteristics of this as yet rather vaguely understood phenomenon (the riddle of the icterus index 166) are as follows (1) As the icterus index ascends in the absence of a direct positive van den Bergh reaction, it is halted

in its rise at 16.6 while at this point the quantitative bilirubin increases sharply to disproportionately high levels. (2) As the icterus index rises from 16.6 to 30 a definite downward loop in the quantitative bilirubin takes place which may continue downward with the rising index, or may recover from its fall to regain at 30 approximately the value it had attained at 16.6. (3) In familial jaundice, pernicious anemia, and in the newborn the rise of the icterus index above 16.6 is usually accompanied by the development of an anomalous type of direct positive van den Bergh reaction, an immediate golden re-entration (instead of the typical reddish or amber color). In lobar pneumonia, however, should this golden reaction actually be present, it is completely masked by the invariable presence of the reddish direct positive reaction. (4) The negro race has not as yet been found to exhibit this phenomenon. Thus far it appears to be associated with icterus only in the white race. (5) Icterus ascending with a direct positive van den Bergh reaction does not exhibit the 16.6 phenomenon in its complete form, for either the halt at 16.6 is prolonged (as in many fatal cases of lobar pneumonia in the white race) or else as 16.6 is passed, no subsequent downward loop in serum bilirubin content is manifest (except in lobar pneumonia in the white race). The icterus of lobar pneumonia in the white race then exhibits the same characteristics in its changes as are exhibited in the type of icterus known as "hemolytic" except for the fact that the van den Bergh reaction is direct positive.

The phenomenon just described is undoubtedly the crucial factor in the consideration of, and approach to the problem of bilirubin therapy in lobar pneumonia. It is also quite obvious that should intravenous injections of bilirubin (in 4 per cent sodium hydroxide) effect a change in the icterus index zones and thereby possibly affect the outcome, unless it be used with all the data at hand, it might as readily result in a fatality in a case which would otherwise have recovered spontaneously, as it might induce recovery in an otherwise fatal case, since depending on the efficiency of the fluid mechanism, it is essential to pass the icterus indices 10 or 16.6 and in case 30 should be exceeded to reach 100. Exactly how much pigment is necessary to force these icterus index zones remains to be determined. The explanation of why the zones should signify what they seem to is by no means clear, but it is probable that at the icterus indices 10, 16.6, and 100 some change takes place in the physical state of bilirubin as it concentrates in a serum medium, manifested by marked disturbances in the relation of the quantitative bilirubin to the icterus index at these points, and due to changes in the size of suspended aggregates of free nascent colloidal bilirubin, which does not give any reaction in the aqueous (direct) van den Bergh test rather than to the bilirubinate which gives rise to the direct positive reaction, and it is probable that these two forms are present together. Although pure bilirubin is now being used routinely by several individuals intravenously as a liver function test only up to 1 mg. per kilo of body weight, it is indicated by Emerson¹⁰ that it could be used in greater amounts without any harmful effects.

Barjot,² Castellanos y Gonzales,⁶ and Ziegler³⁴ have used bile salts intravenously in spite of their known toxicity with apparently very favorable results.

in pneumococcus infections, and it may well be considered whether or not the bile salts play any rôle in the icterus index zones. This conjecture, however, cannot be confirmed or refuted until a suitable simple method is devised for the quantitative determination of the bile salt content of whole blood,²⁷ ²⁸ ²⁹ for, unlike bilirubin, they are found in normal red cells²⁷ as well as in the plasma.

RATIONAL

I *The Icterus Mechanism*—Analysis of the cases observed since the beginning of this study indicates that approximately 30 per cent of hospitalized patients having lobar pneumonia do not form any fluid in their pleural cavities and face a mortality of from 75 per cent to 80 per cent. Recovery in such cases appears to depend exclusively upon the movements of their serum icterus. Obviously bile-solubility should be analyzed as fully as possible. Atkin¹ found that if pneumococci were cultured so that they contained no auto-lysis they were not bile-soluble, and concluded that bile solubility was but an acceleration of the normal autolytic process of these organisms, analogous to the action of bile on any autolytic process. In the consolidation of lobar pneumonia, which is the most striking feature of localization in the human organism in response to pneumococcus invasion, the outstanding biochemical phenomenon is likewise autolysis. Cleavage products are much in evidence, arising from this consolidated area, leucocytic enzymes playing a prominent rôle in their production³⁰ although they are inhibited in this rôle by blood serum¹⁰ which also inhibits autolysis.¹⁷ Pneumonic sputum contains peptones, proteoses, and amino acids and acquires a marked peptolytic activity after "crisis"¹¹, albumoses appear in more than 60 per cent of the cases in blood or urine³¹ during and after the fastigium, the urine contains an excess of organic acids¹⁴, there is an increase in noncoagulable nitrogenous substances in the blood³² and after crisis, the serum contains an enzyme acting specifically on pneumococcus protein accompanied by an increase in serum crep-tase³³. Weiss³² found by lung punctures that there was insufficient undigested pneumococcus protein in consolidated areas to produce any anaphylactic phenomena in sensitized animals. Lung punctures have also shown that in cases evolving favorably no living pneumococci could be obtained after the fifth day²¹ although clinical recovery did not take place until later. It appears, then, that the organisms in their pulmonary foci are undergoing autolysis, hence their two fractions, the haptene (specific soluble substance of Avery and Heidelberger) and the common pneumococcus protein are split from one another,³⁴ the former neutralizing artificially injected specific antibodies and the latter stimulating the formation of natural antibodies specific only for pneumococcus protein or its cleavage products,¹⁷ and type specific antibodies, of spontaneous origin, would hardly be expected to appear in any appreciable concentration.

It has been found that pneumococcus autolysis at first produces a highly toxic proteose, but that if this autolytic process is carried on beyond the proteose stage, all toxic properties disappear.¹⁶ It is significant that Rosenow²¹ treated 200 cases of lobar pneumonia by the intravenous injection of autolyzed

pneumococci (sufficiently autolyzed so that most of the toxic substance had disappeared) with a mortality of 3 per cent in 95 cases treated within forty-eight hours, and of 11 per cent in 105 cases treated on or after the third day.

The question now arises: 'What particular constituent of bile accelerates autolytic processes?' and specifically: 'What constituent might accelerate the autolytic process of lobar pneumonia beyond the stage of toxic cleavage products?' Titum¹ found that whole bile was more effective, although less penetrating than the bile salts alone, in accelerating tissue autolysis, and also observed that bile with its bilirubin content removed was less effective than whole bile. It is generally assumed that the bile salts are the substance active in producing pneumococcolysis, but Kelly² and Sellards³ have found that whole bile is more reliable, since different strains of pneumococci vary in their susceptibility to lysis more with the salts than they do with whole bile. Sellards could obtain no evidence that bilirubin, known to be cytolytic in the presence of light, was at all pneumococcolytic in a 1 per cent alkaline solution in the test tube.

Experiments in animals frequently give results different from those observed in the test tube. Koslowski⁴ has pointed out there are present in bile numerous unconjugated higher soaps which are far more pneumococcidal *in vitro* than sodium taurocholate or sodium glycocholate. On the other hand Lamar⁵ in showing that pneumococci could be destroyed by sodium oleate, observed that although sodium linolate and sodium linolenate were more effective in this action in test tube experiments, the reverse held true in animals for serum completely inhibited the action of the more unsaturated soaps, and the greater the degree of unsaturation the less was the protection afforded the animal. The evidence that the pigment bilirubin is primarily involved in the relation of jaundice to the outcome in lobar pneumonia is derived entirely from the study of patients, and is sufficiently consistent to suggest, as a provisional hypothesis, that the autolytic process of lobar pneumonia is accelerated by a change in the physical state of bilirubin associated chiefly with "the riddle of the icterus index 16.6," whereby a form of the pigment evolves which acts as a catalyst on this autolytic process, as well as acting destructively on all autolysin containing pneumococci, that until and unless this change takes place the pigment has no favorable effect upon the disease or the organisms, except in cases where it is acting in conjunction with the effusion mechanism. It is of interest that from many medical centers during the influenza epidemic of 1918 type specific pneumococci were frequently reported to be bile-insoluble,¹⁷⁻²⁴ and therefore not subject to autolysis.

In the negro race the findings indicate that at the icterus index 100 a phenomenon analogous to, but not identical with, that which takes place in the white race at 16.6, occurs. White patients who exceed the icterus index 30 develop curves which may be interpreted only as negroid reactions. From the phylogenetic point of view it is possible that these curves do not always indicate negro ancestry (although such ancestry was disclosed in the only case in which inquiries were made) but may often be due to other racial mixtures.

II *The Fluid Mechanism*—In approximately 70 per cent of the cases observed in this study there was evidence of the presence of a fluid pleural exudate, and in this group the mortality was only 15 per cent. Effusions were often demonstrable before the icterus index had dropped to normal, thus affording data as to the zone entered and forecasting the outcome. However, in those cases found presenting icterus indices below 10 when first seen, no data were available to show whether the 11-15 zone had been entered or not, and in such cases the outcome could not be forecasted with accuracy. In this latter group the van den Bergh reaction was closely followed, and danger of death remained as long as the direct positive reaction persisted.

A description of the pulmonary lymphatic system makes it possible to understand the mechanics of pleural fluid formation. Gage¹¹ states that the lymphatic drainage of each lobe is so constructed that the peripheral portion of the lung parenchyma is drained toward the pleural surface, whence collecting lymphatic channels run subpleurally to the hilum. It is probable that this system cares for the drainage of a large portion of the alveoli, for they are not reached by the deeper or peribronchial and perivascular system.¹³ In the presence of an inflammatory process, shutting off the lymphatics of a lobe, the slightest change in the permeability of the walls of these lymphatic channels would result in a fluid pleural exudate. Furthermore, Gorecki¹² points out that carbon particles injected intratracheally in dogs later are found on the pleural surfaces, a finding again consistent with the operation of this peripheral pulmonary lymphatic system. Should consolidation take place rapidly fluid mobility would be checked by the dehydration of the lobe involved, and no fluid could form in the pleural cavity.

This fluid mechanism seems analogous to drainage, coupled with the extraordinary properties of the constituents of pleural effusions contributing to the destruction of pneumococci. Often when such drainage occurs promptly all that can be found in the chest is a small effusion persisting for such a short time that it may easily be completely overlooked, while others, due to their long duration, finally compel the clinician to diagnose them, although without cell studies they may readily be confused with the effusions of tuberculosis. The successful operation of the effusion mechanism appears to depend largely on three factors: (1) unimpeded autolysis of pneumococci, (2) phagocytosis and the action of leucocytic enzymes, (3) the bilirubin content of the fluid (dependent on the serum level). The greater number of these fluids are sterile, even during the acute course of the disease, adequate evidence of their pneumococciostatic or pneumococcicidal power. Duyck,⁸ working on pneumonia in the natives of the Belgian Congo, found that a few drops of such pleural fluid rendered a broth culture of virulent pneumococci innocuous to susceptible animals with many times the ordinary lethal dose.

The rôle of white cells (polynuclears, plasmatocytes, monocytes) in the successful operation of the effusion mechanism, correlated with the bilirubin content of the fluid appears of paramount importance, for favorable cell immigration and emigration was observed only in the fluids of higher bilirubin content. Since the fluids obtained in the Boston City Hospital series were

submitted to Drs. Scott and Forkner of the Thorndike Laboratory for cell studies and to Dr. Finland for serologic studies, the detailed description of this part of the work will be left to them. Cells were sufficiently numerous in fluids from recovery cases to cause them to appear turbid grossly like empyema but microscopically showing active living cells in vital preparations. The various phases of cell activities observed in successive taps and the changes associated with empyema will be discussed by Drs. Scott and Forkner. During the recession of the serum icterus the bilirubin content of the fluids remained high and receded very slowly indicating the slowness of reabsorption from the pleural cavity thus affording a possible means of estimating the maximum intensity of the icterus previously attained.

In tapping for pneumonic effusions it was often noticed that a marked drop in the fever followed more frequently than could be ascribed to coincidence. None of the cases tapped early continued prolonged febrile reactions during convalescence. On the other hand in many cases tapping seems not at all necessary.

The site for tap is obviously wherever the signs of fluid are found often the costophrenic angle in the posterior axillary line and frequently more mesially posteriorly in the midscapular line or closer to the spine. Interlobar pockets are best diagnosed by x-ray. It should be borne in mind that the fluid is usually in a thin sheet between lung and chest wall,²² and that a shallow tap is necessary. Tapping, if contemplated for diagnosis or for the study of fluids should be done as early as possible, since many fluids do not persist longer than a few days. The duration of fluid exudates in the pleural cavity has been stated by Gorecki to be proportional to their colloid content, since the digestive action of enzymes is required before such substances can leave the pleural cavity. Withdrawal of fluid soon after its formation removes these colloids (proteins), thus facilitating reabsorption and reducing to a minimum residual adhesions from the organization of fibrin.

A dry tap, unless repeated at different sites, is not conclusive evidence against the existence of fluid but the obtaining of it in any amount is positive evidence for ordinarily the pleural cavity contains only enough moisture to lubricate the pleural surfaces and not enough to be obtainable by tap.

A chest needle, fitted directly on a 50 to 100 c.c. syringe, has proved most satisfactory, since the resistance to the progress of the needle point may be palpated by the rigidity of the instrument, and it is quite easy to detect the entry into the pleural cavity, contact with the diaphragm, or the yielding touch of lung surface. Care should be taken to make all movements in line with the shaft of the needle, even when searching laterally in the space between lung and chest wall. With the proper use of novocain chest-tapping caused little or no pain, except in an occasional patient with a thick chest wall. The asepsis was essentially the same as that used in routine lumbar puncture.

Normal icterus indices, when found after the fourth or fifth day of illness, have in the adequately studied cases been significant of the existence of an effusion, but in such cases a prognosis cannot be made on the basis of the

normal index Beinheim¹ found in a series of cases that those with normal icterus indices did not die, and although her findings are confirmed by a large number of noninfluenzal cases in this series, there have also been several discouraging exceptions. In the normal icterus index group the van den Bergh reaction has proved the test of value, for these patients are decidedly not out of danger until even the weakest possible direct positive reaction has disappeared. Since such cases are often not studied until late in the course of the illness, no data are available as to whether or not they had previously exceeded the icterus index 10 without examination of the pleural fluid.

SUMMARY

Serial studies in 224 cases of lobar pneumonia by the daily determination of the icterus index, van den Bergh aqueous (direct) reaction, and the quantitative serum bilirubin estimation have demonstrated that serum icterus manifested in one or all of the three tests is pathognomonic of the disease. The changes in intensity and character of this icterus may be closely correlated with the events of the pneumonic fastigium; their interpretation depending on (1) the determination of the maximum icterus index attained and (2) the determination of the presence or absence of a fluid pleural exudate arising from each lobe involved. In the absence of such fluid the icterus index tends to oscillate upward; in the presence of fluid it tends to return to normal. Fluid pleural exudates were found or indicated in 70 per cent of the cases studied, and in this group the mortality was 15 per cent. In the 30 per cent without fluid however the mortality was 75-80 per cent. The total series mortality was 37.2 per cent.

In the absence of fluid, in the white race, the mortality was 100 per cent with icterus indices up to and including 16.6. In cases entering and not exceeding the icterus index zone 18.7-30 there were no deaths and the day of crisis corresponded with the attainment of the maximum icterus index. Following the drop of the index during crisis, it remained above normal for several days. A corollary study of icterus in other diseases and conditions implies that in ascending jaundice the icterus index 16.6 is a biologic constant in the white race, and that in the absence of a direct positive van den Bergh reaction (hemolytic jaundice) a change in the physical state of bilirubin occurs as it is passed, this change manifesting itself by a downward loop in serum bilirubin as the index rises in the zone 18.7-30. In lobar pneumonia, although the van den Bergh reaction is direct positive, this same phenomenon takes place, and appears to be associated with recovery.

In negroes, in the absence of fluid, the icterus index 16.6 is of no significance, and recoveries occurred only when the index 100 was exceeded. When, in the white race, the icterus index 30 was exceeded, recoveries then occurred (by true crisis) only if the index 100 was attained, thus manifesting a reaction essentially negroid in character.

In the presence of pleural fluid (70 per cent of cases), in the white race, no deaths occurred in the icterus index zone 11-15. In many cases, however, constantly exhibiting icterus indices at or below 10 during the period of

observation it could not be ascertained whether or not they had previously entered this zone and the estimation of the outcome depended on the determination of the changes in the van den Bergh reaction recovery being indicated by the confirmed disappearance of the direct positive reaction. In the negro race insufficient cases have been observed to make definite conclusions when fluid is present although no deaths have as yet been observed in the icterus index zone 11-30.

The prognostic value of the study of the icterus in lobar pneumonia should not perhaps be unduly stressed since each case exhibits its own individual characteristics and often the trend of the icterus is not discoverable until the clinical indications of the outcome are clearly manifest. Frequently however such a study furnishes a means for the estimation of the outcome in doubtful cases.

The summation of the findings relating to the pneumonic icterus clearly indicates that a therapeutic trial of bilirubin is the logical conclusion of the investigation particularly since the pigment is believed to be nontoxic when injected intravenously. Although the analysis of the icterus of lobar pneumonia may seem complex because of its relation to the presence or absence of fluid and because of the differences between the white and black races the application of these findings to the therapeutic test is simple enough and may be stated as follows:

1. In the white race inject a quantity of bilirubin sufficient to cause the icterus index to pass 16.6 but not to exceed 30 regardless of the presence or absence of fluid. (It is possible that to exceed 30 may not be detrimental in pure Caucasian stock.)

2. In negroes, and when negroid reactions occur in the white race the icterus index 100 must be attained.

3. By preliminary studies correlating the serum icterus with the physical findings the addition of surplus bilirubin may be contraindicated in many cases.

4. Additional data on the bilirubin content of pleural fluid obtained early in the course of the disease in its relation to the outcome should be secured.

The writer wishes to express sincere appreciation of the thorough and scientific manner in which much bacteriologic data were obtained by the members of the Pneumonia Service at the Boston City Hospital, and for their splendid cooperation in studies pertaining to the proper evaluation of some of the pathologic phenomena of lobar pneumonia. The results with specific serum therapy were favorable.

Studies on the physical chemistry of pure bilirubin, obtained from the Chemisch Pharmazeutische A. G., Bad Homburg Werk Frankfurt, a. M., Germany, in so far as they have as yet been carried out are confirmatory of the significance of the icterus index constants encountered in lobar pneumonia from the point of view of colloid chemistry.

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THE EFFECT OF ULTRAVIOLET IRRADIATION ON GLUCOSE SOLUTION^{*,†}

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THE effects of ultraviolet radiation on glucose solutions as referred to in an earlier^s paper prompted a further investigation as a preliminary to the study of the effects of the procedure on carbohydrate metabolism.

Berthelot and Gaudechon^r found that 10 per cent glucose was quantitatively decomposed to carbon monoxide, methane and hydrogen after ten hours' exposure to a quartz mercury vapor lamp. Neuberger^r on the other hand found no change in rotation after exposing 10 per cent glucose solutions to sunlight over a period of two months or longer but the solution became less dextro-rotatory after three days if ferrous sulphate was added. Euler and Lindberg^r irradiated a 10 per cent glucose solution with a quartz mercury vapor lamp at 75° and found that gases were evolved after two hours, after twelve hours the gases were identified as methane, hydrogen, carbon monoxide and carbon dioxide. Lowry and Courtman^r found that irradiation for thirty minutes with a quartz mercury vapor lamp did not accelerate isomeric changes.

A possible explanation of these conflicting reports is the lack of standardized conditions of time of exposure, source of radiation and voltage, the distance of the solution from the source of energy, the temperature of substance as well as its P_H , and the presence of salts or catalysts, every one of which is variable and yet determines to some extent the course of the reaction.

The object of the present investigation was to determine the nature of the change in glucose solutions after irradiation, since most reports indicate some changes. It was, at first intended to use some one of the uric acid methods for analysis, since in this way a constant standard could be employed, i.e., the amount of uric acid reduced. Due to the variability of the reduction of phosphotungstic acid by dilute glucose solutions the standard methods were out of the question (Table IV)^s. These methods were modified by using different concentrations of Na_2CO_3 , $NaOH$, and other alkalis but the results were unsatisfactory. Micromethods for blood sugar were not considered because these entailed determining the percentage of change in reducing power and not a constant standard.

In a few preliminary experiments it was noted that the total reducing power of the glucose solution did not change, whether it was irradiated or not, according to the Folin-Wu¹ micromethod, Benedict^r micromethod, or Shaffer and Hartmann^r method. Since the mechanism of the oxidation of glucose by alkaline copper is not perfectly understood, it is logical to suppose that the

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glucose might be so rearranged and slightly decomposed that the mixture produces the same total reduction as the freshly prepared glucose solution. If now we determine the reduction of the mixture (glucose alkaline copper) at intervals of one minute and plot a curve of this reaction a mixture of decomposition products giving the same total reduction would perhaps show a different curve of reduction.

The curve of the reduction of alkaline copper by glucose was worked out as follows: 5 cc. of the Shaffer Hartmann citrate reagent and 5 cc. of glucose solution (Merck's c.p. dried 24 hours at 85°) containing not more than 20 mg. of glucose were pipetted into a large test tube and placed in a boiling water-bath for a certain time (indicated in Table 1) cooled, acidified with 5 cc. of H_2SO_4 and titrated with 0.005 $\text{Na}_2\text{S}_2\text{O}_3$ starch used as indicator.

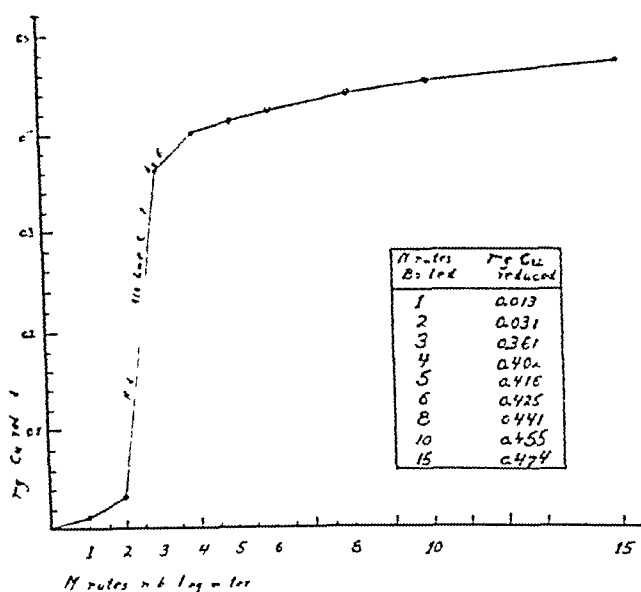


FIG. 1

Results of the averages of at least four determinations are shown in Fig. 1. Titration agreed surprisingly well considering that we stop a reaction while proceeding at a fair rate of speed. After three minutes boiling, the titrations checked within 0.1 cc. Reduction occurs as soon as the tube and contents have reached the temperature of the water-bath. The first part of this reaction, the first three minutes, is the stage in which we would expect to find a deviation for a decomposition mixture. This was accomplished by reducing the temperature.

The experiment was repeated, except that the temperature was maintained at 70° to 75°. This time in the water-bath as indicated in Fig. 2. There is a "latent" period before any noticeable reduction occurs.

* CuSO_4 —5 gm. per liter. Na citrate—16 gm. per liter. KI —10 gm. per liter. KIO —0.7 gm. per liter. K_2CrO_7 —18 gm. per liter. The copper is reduced by the glucose and on acidifying iodine is liberated which oxidizes the cuprous oxide according to the equation $2\text{Cu}^+ + \text{I}_2 = 2\text{Cu}^{++} + 2\text{I}^-$. The excess iodine is titrated with thiosulphate.

Aqueous solutions of Merck's c p Glucose dried 24 hours at 85° (solution prepared the same day as used) were irradiated in a fused quartz flask (5 mm in thickness) at a distance of one inch (at this distance there was uniform diffusion throughout the flask) with a water cooled Kromayer quartz mercury arc lamp at room temperature (21°-23°)

TABLE I
DEGREES OF REDUCTION

PER CENT GLUCOSE		TIME IRRADIATED				
		15 min	30 min	60 min	90 min	5 hr
0						
0.1	0.103		0.103	0.107	0.103	
0.5	0.52		0.49	0.49	0.49	
1.0	1.07		1.14	1.07	1.11	
5.0	5.23	5.2	5.26			5.26
10.0	10.71	10.74	10.74			10.76
15.0	16.16					16.08
20.0	21.41					21.48
50.0	55.09					55.29
50.0	55.36					55.23

Solutions 0.1 per cent, 0.5 per cent, 1.0 per cent were made up fresh and three drops of 0.2 per cent NH_3 added before measured.

Solutions 5.0 per cent to 50 per cent were made up and set in refrigerator for two weeks or longer.

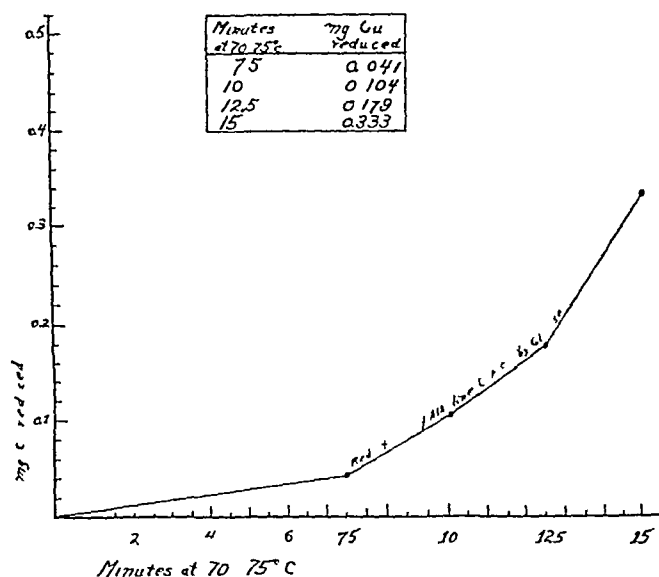


Fig 2

The reduction curve, or parts of the curve, was determined for the following

- 1 0.1 per cent irradiated 30 minutes
- 2 0.1 per cent irradiated 60 minutes
- 3 0.1 per cent irradiated 90 minutes
- 4 1.0 per cent irradiated 30 minutes
- 5 1.0 per cent irradiated 60 minutes
- 6 1.0 per cent irradiated 90 minutes
- 7 1.0 per cent irradiated 3½ hours

Complete curves were worked out in Cases 1, 2, and 3 but are not reproduced because they agree in every particular with the curves in Figs 1 and 2

In Cases 4, 5, 6, 7 only the most prominent points of the curves were plotted, namely, boiling water bath for four and fifteen minutes and at 70° to 75° for three, six, and nine minutes. In none of these cases was there any divergences from curves shown.

It was thought that perhaps the reagent was too strongly alkaline to detect any slight change that might be present, so the experiment was repeated (the most prominent points of the curve being plotted) using the original citro carbonate reagent to which had been added 0.1 M boric acid. This solution slowed the reaction, but the curves of the normal and of the irradiated solutions did not differ. Two tenths M boric acid was now added to the original citro carbonate reagent which further slowed the reaction but did not produce any change in the form of the curves.

By the method employed we can determine quite accurately any change in reducing power that may be produced in a given solution, but in view of the negative results there was a question whether any change had been produced in the glucose solutions, so polariscopic measurements were made to determine the rearrangement.

Fresh solutions of glucose in distilled water were prepared as described above irradiated for thirty, sixty, and ninety minute intervals and the rotation determined after addition of 3 drops of 0.2 per cent NH_4OH . Results are shown in Table I. It is obvious that there is no rearrangement in any of the solutions, all readings being within the limit of error. No change in optical rotation is observed in 20 per cent glucose after five hours' irradiation.

Two samples of 50 per cent glucose solution showed no change in rotation after five hours' irradiation, although in one sample gas bubbles were produced after four hours.

CONCLUSIONS

By the methods employed, reduction of alkaline copper solution and polariscopic measurements, it was impossible to determine any change in glucose solutions when irradiated with quartz mercury vapor lamp.

Thanks are due Dean A. H. Daniels of the Graduate School, to the committee on Scientific Investigation of the American Medical Association, and to the Council of Phi Rho Sigma Medical Fraternity for financial assistance.

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STUDIES IN THE ALIMENTARY CANAL OF MAN*

IX THE CALCULATION OF GASTRIC VOLUME †

By W. A. SOMMERFIELD, M.D., CLEVELAND, OHIO

INTRODUCTION

IN THEIR studies upon gastric reaction patterns Miss Kuenzel and Doctor Todd, in a series of papers have demonstrated the effect of different stimuli upon the behavior of the stomach. They have shown for instance, that, following buttermilk, the roentgenographic shadow is longer, broader, and of larger area than after a milk meal of the same bulk and under the same conditions. They have shown that warming the abdominal wall or administration of a hot milk meal is followed by a shadow of less dimensions and smaller area than that resulting from a milk meal of the same bulk at 70° F administered without application of heat.

Continuing this important line of investigation the authors mentioned have shown that shadow area is modified in the same subject following the administration in equal bulk of water or lactic acid and after application of cold. Further they have been able to demonstrate that the characteristic shadow area is exaggerated by previous administration of a preliminary meal, a curious phenomenon which has been termed facilitation.

Comparison of linear dimensions of gastric shadow has its defects for the method of measurement must necessarily utilize as measuring points, standardized arbitrarily selected sites upon the shadow contour. A standardization of this type can take no account of the variations in disposition of the stomach which the presence of the other abdominal viscera induce by altering its tilt or position from time to time and even during a serial roentgenographic study. It is also difficult to discount the fluctuations in shadow pattern necessarily accompanying peristalsis and purely local changes in the Magenblase.

As a check upon the interpretation of linear dimensions Miss Kuenzel used the planimeter record of shadow area but apart from the error of 6 per cent which she claims is inevitable in these records⁸ the shadow area, though permissible as an index of volume, gives no real assurance of the volume which, after all, is what we really seek to obtain.

Linear dimensions and area of roentgenographic shadow are of value, utilized in the manner and for the purpose described by these authors, in whose hands the records so obtained have permitted a quantitative interpretation of roentgenoscopic observations on gastric activity. But these methods have been

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employed temporarily pending a time when more reliable or appropriate technical devices should be made available. It is my purpose therefore to describe an alternative and possibly a more satisfying method of analysis of the roentgenographic record. I shall discuss the technique, apply it to the elucidation of gastric problems and seek to give the method its validation in examining critically the results obtained in the light of information secured along other lines.

CURRENT ESTIMATES OF STOMACH CAPACITY

Observation on adult stomach volume have been made by Luschka, Ewald, Ost and Kelling.

Luschka used dead stomachs, blew them up moderately so as not to stretch their walls, drained them of contents and then filled them with water. In seeking to determine gastric form by filling the organ in situ with plaster, Luschka recognized that he had also an alternative method of obtaining volume.

Ewald² used Luschka's method but attained no more satisfactory results because he merely forced water under pressure into the dead stomach.

Ost³ modified this technique to some extent in permitting water to flow by its own weight alone into the stomach instead of by pressure from behind. But Ost also investigated the living organ, filling it in the same way by allowing water to flow in until the patient complained of pain and then abstracting the water by suction or forcing in an under pressure with the same end in view.

Kelling⁴ forced air into the living stomach to a prearranged pressure and then detaching the stomach tube from the pump collected the air regurgitated.

All these methods are so obviously crude and inaccurate that it is hardly necessary to dwell on them. In summary they are indirect measurements made during life and direct determinations after death. Both depend essentially upon the introduction of fluid under artificial and uncontrollable conditions. Other authors have pointed out that the amount of gas or liquid escaping through the pylorus or lodging in the esophagus is not ascertained. There are objections even more significant than these in the living. We have as yet no precise information concerning the reflex effect of introducing a rubber tube into the stomach. Motility, secretion and size, we find, may be affected by direct reflex or cerebral stimulation. There is probably a marked complication from the secretion of gastric juice and our roentgenoscopic observations warn us that this secretion may be both considerable and rapid.

Measurement of volume in the dead stomach has still more obvious defects, for the organ is atonic or, if the cadaver be embalmed, may present bizarre forms in shape or volume as was demonstrated long ago by Cunningham¹. As a matter of fact we may eliminate at once the estimates based upon measurement of autopsy specimens or those older records drawn from the determination of volume upon dead organs embalmed by arsenic alcohol or other reagents before the introduction of formalin.

Nevertheless gastric volume is currently conceived to lie somewhere between 1500 and 3000 cc. Vierordt⁹ has gathered together the various estimates which are summarized in Table I.

Taking a series of 10 stomachs at random from adult male bodies embalmed by a mixture of alcohol, carbolic acid, and glycerin with about 5.45

TABLE I
ESTIMATES OF STOMACH CAPACITY IN CC OBTAINED BY INTRODUCTION OF FLUIDS

	DEAD		LIVING	
	AIR	WATER	AIR	WATER
Luschka		Male 2500 to 2600 Female 1750		
Ewald	250 1600		1600 1700	
Ost			1830 2700	2267 2533
Rosenheim			1700	
Kelling			587 1300	1300 1700

per cent of formalin I have obtained the results presented in Table II. The organs after excision were ligated on the duodenal side of the pylorus and a pursestring suture was tied around the esophageal orifice into which a nozzle was inserted. The stomach was carefully filled with water which was then emptied into a graduated cylinder. The average volume of stomachs 2 to 10 inclusive all showing formalin contraction was 563.5 cc. So small is the figure in comparison with that currently quoted that one might reasonably doubt the validity of the method which is undoubtedly complicated by the formalin injection. A single specimen of flaccid stomach gave a capacity of 1263 cc but even this figure is smaller than most accepted averages.

TABLE II
CAPACITY OF ADULT MALE STOMACHS IN CC REMOVED FROM FORMALIN EMBALMED BODY AND WATER FILLED

NO		NO	
1	1263	6	405.5
2	684.5	7	405.5
3	474	8	629
4	541	9	609
5	473	10	850
Average 633.45 cc			

THE MATHEMATICAL DETERMINATION OF STOMACH CAPACITY

Having failed to obtain anything like the usual figure by filling the dead stomach with water and suspecting current estimates of exaggerating the actual capacity we decided to seek a method of determining volume from the roentgenographic picture of the living stomach. For this purpose the ordinary anteroposterior roentgenogram is not sufficient. We must also observe the gastric shadow in the lateral roentgenogram. Fortunately a number of such records had been made by Doctor Todd and Miss Kuenzel. From these, one notes that the stomach after a small meal is a cylindrical tube slightly flattened from before backwards. The flattening varies somewhat even in the same individual from time to time but for practical purposes, within the lim-

its of determination possible this variation may be ignored. We then measured the shadow of a barium filled stomach projected upon the roentgenoscopic screen and having equalized as well as possible, the distance between stomach and screen in both lateral and anteroposterior postures, found the gastric tube shadow just below the Magenblase 85 mm broad in both anteroposterior and lateral roentgenograms. Though this line of investigation must be methodologically unsatisfying it taught us, at least that the stomach after a small meal may for practical purposes, be considered a cylindrical tube and thus our expectation that the roentgenographic shadow could be used as a basis of estimating stomach volume received encouragement.

It has already been stated that hitherto, in this laboratory, the area of gastric shadow has been measured by the planimeter. But it might have been done more accurately though with greater expenditure of time, by the application of Simpson's rule. I do not purpose to trace the derivation of this rule from the analytic expression of a parabolic curve. For that the reader should refer to any favorite exposition of the calculus. There is a very clear account in Gibson's little volume.¹ Simpson's rule is the following: if an area under a curve of unknown analytic expression is to be determined, divide this area into an equal number of strips by equidistant ordinates. Then find the sum of the extreme ordinates, plus twice the sum of the odd ordinates, plus four times the sum of the even ordinates. Multiply this quantity by the fraction obtained by taking one third of the distance between ordinates.

Clearly a calculation so involved as that just enunciated is not worth while in practice unless one can be fairly sure of its approximation to a true valuation of volume, especially as the planimeter reading can be made in a fraction of the time. In our preliminary survey of shadow areas made upon several hundred roentgenograms, when we could hope only for rough approximation, the planimeter served admirably. By its help Miss Kuenzel and Doctor Todd have differentiated size of shadow area under different conditions as I have already stated. These investigators have therefore been able to forecast more or less roughly the probable differences to be found in stomach volume under different experimental conditions. The application of Simpson's rule becomes imperative to complete our study, now that it is possible to make the necessary corrections in dimensions of shadow area.

AN ILLUSTRATIVE EXAMPLE

With the human stomach as our example of a tube of irregular contour, and a tracing of the projection of its outline on a roentgenogram as our basis for calculation, assuming the tube to be circular in cross-section diameters at equidistant intervals are measured off and the areas at these levels calculated from the formula $\frac{1}{4}\pi D^2$

When the distance between ends of summit of Magenblase and lowest point of greater curvature, measured parallel with the vertebral column, is divided into ten equal intervals and the capacity calculated by Simpson's rule, we find that the result differs by only 4 c c from the capacity computed when twenty such intervals are used. Hence we have restricted our calculations to the ten-interval basis.

To obtain ten equal intervals, eleven ordinates are drawn at right angles to the parallel just defined. The first and eleventh of these being tangents to the gastric outline, give areas of zero value hence there are nine areas to compute. The stomach volume is then found by adding twice the sum of the areas derived from the odd ordinates to four times the sum of the areas derived from the even ordinates and dividing the total by one third of the distance between two successive ordinates.

THE ARTIFICIAL TEST STOMACH

In the course of the work a suggestion was given that perhaps more accurate results might be obtained if one drew the ordinates perpendicular to the axis of the stomach. In order to compare the probable reliability of the

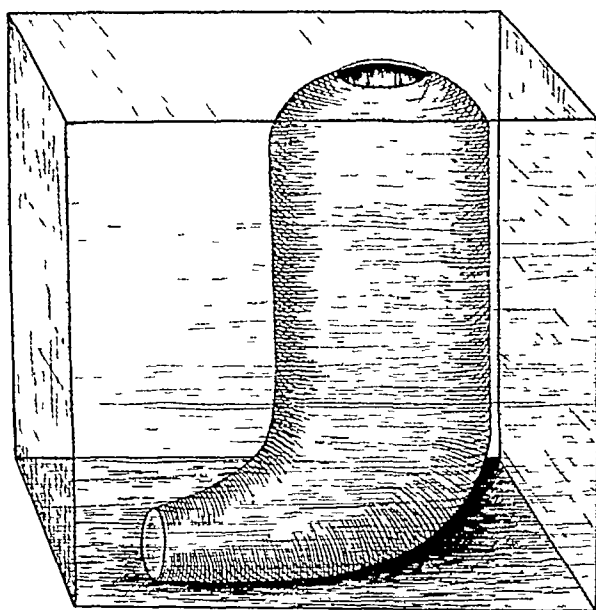


Fig 1—Plaster mould manufactured from model of artificial stomach. See text.

suggested technique with the method already described an artificial stomach was cast in plaster. This rough model of an average stomach was scraped down to even dimensions (see Fig 1) and its volume measured. From it, a plaster mould was constructed and the two halves fitted exactly together leaving a small opening above to permit the mould to be filled with water. Before fitting the halves together the interior was thoroughly coated with shellac so that it would be water-tight.

From the cast model a set of calculations were made by each method. Finely adjusted calipers graduated in half-millimeters were used to determine the diameters at the different levels. All measurements were checked twice. The first method detailed above gave a volume of 580.56 cc and the suggested alternative method gave one of 493.81. Then the mould was filled with water and emptied into a 1000 cc graduate cylinder. The actual vol-

time was found to be 580 c.c. Not only does this prove that the first method is correct but that it is accurate within experimental and calculable limits.

The source of error in the suggested alternative method lies in the fact that measurements based on a curved axis do not apply to a formula calculated on Cartesian coordinates. In order to develop a formula for the curved axis of the stomach from Magenblase to pylorus one would have to use polar coordinates and conical sections. This would be far too cumbersome and there is no expectation that it would prove to be more accurate than the simple application of Simpson's rule for plane surfaces to the calculation of volumes.

THE CORRECTION FOR OBLIQUITY

The technique just described leaves out of account the distortion of shadow outline produced by obliquity of the stomach. Hence a correction must be made in the ordinates actually measured on the tracing of the roent-

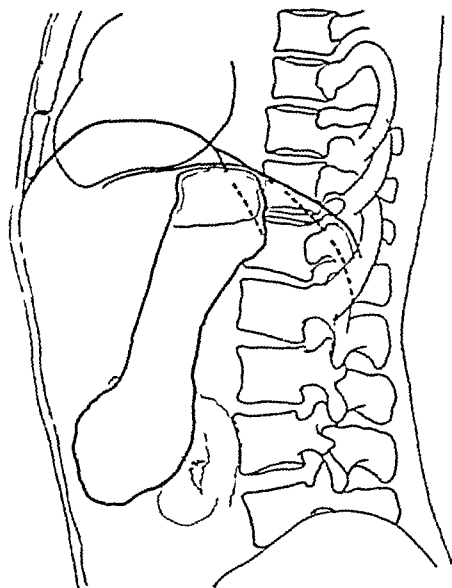


Fig. 2.—Lateral roentgenogram showing oblique relation of stomach to anterior abdominal wall

genogram. Inasmuch as the upper part of the stomach lies further from the photographic film than the lower part its shadow area is correspondingly greater. Hence we must know the approximate forward inclination of the organ upon what is called the stomach bed. For this the lateral roentgenogram is advisable though not absolutely necessary since the slope of stomach may be sufficiently accurately calculated by considering the stomach itself as the hypotenuse of a right angled triangle of which the side is the vertical height of stomach shadow measured on the tracing parallel with the vertebral column and the base is one half the anteroposterior diameter of the patient at the level of the Magenblase. If this diameter has not been actually taken, it may be assumed to be 35 mm. for a slender male subject and 50 mm. for one of stout (pyenic type) build.

Since the rays spread from the anode in the form of a long cone it follows that the breadth of shadow at any given level will bear the same relation to the actual stomach diameter as the distance of photographic film from the target bears to the distance of that gastric plane from the target. Having obtained and plotted the stomach obliquity towards anterior abdominal wall it is easy to compute the distance of any particular stomach level (or ordinate) from the anode because the distance between target and photographic film is known. The necessary correction of the ordinate measurement on the tracing is then made by simple proportion. When all the corrected ordinate values have been computed one may proceed to the application of Simpson's rule. In order to put this method to a practical examination the following technique was devised. The obliquity of a test stomach was first computed from a lateral roentgenogram (Fig 2). A piece of card cut at this angle is

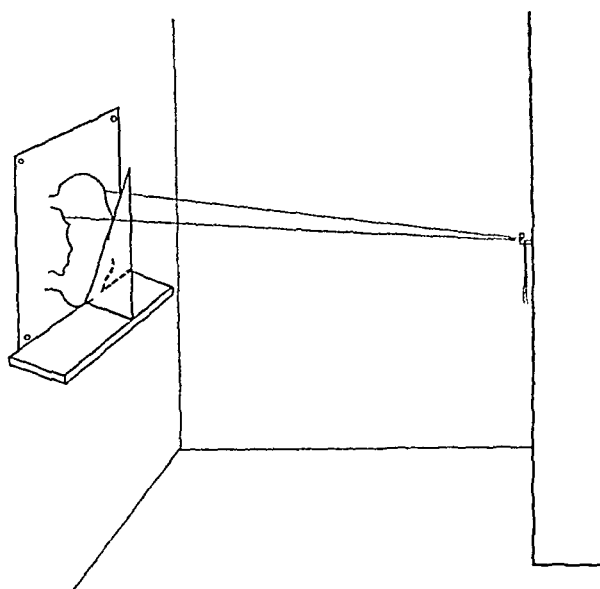


Fig 3—Apparatus for checking computed corrections of shadow diameters (ordinates). Target placed approximately at level of disc between first and second lumbar vertebrae. Strings with needles attached to stomach outline at extremities of an ordinate. Cardboard marker reproducing obliquity of stomach. By a steel rule measurement is made of the distance between the threads at the card hypotenuse.

mounted at right-angles to a stomach tracing, its lower angle just touching the lower margin of greater curvature on the tracing. Two threads representing divergent rays, each having a small needle at its free end, are attached to a support representing the target and placed at the target distance from the photographic film. The needles attached to the threads are then inserted into the traced stomach outline at the extremities of successive ordinates (see Fig 3). The distance between the threads is measured by a steel rule placed against the sloping edge of the card and the observed distance checked against the computed distance.

As an illustration of the use of this method I have measured a series of forty roentgenograms of a single trained and stabilized stomach taken at intervals of twenty seconds after the swallowing of a meal composed of four

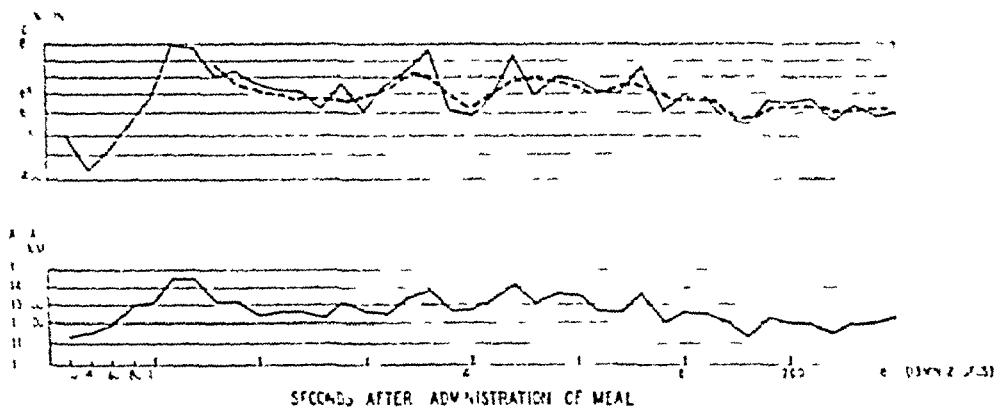


Fig 4—Comparison of stomach volume and shadow area on arithlog paper. Diminution of volume and its rhythmic fluctuations are better seen in the graph of volume than in that of shadow area. Smoothing is obtained by averaging the determinations at successive levels. The first five roentgenograms are of little value for it is only in the sixth that the barium fully canalizes the stomach.

TABLE III
ROENTGENOGRAPHIC STUDY OF STOMACH AFTER 5 OZ BUTTERMILK MEAL

SERIAL NO IN STUDY (WAS)	VOLUME CC	AREA SQ MM
1	541.37	11440
2	467.85	11560
3	507.40	11960
4	566.10	13060
5	636.34	13160
6	701.08	14580
7	784.15	14500
8	695.60	13140
9	710.10	13220
10	673.32	12460
11	658.97	12600
12	653.08	12640
13	610.83	12360
14	676.88	13280
15	603.78	12760
16	675.88	12530
17	715.58	13460
18	772.21	13870
19	607.05	12760
20	593.23	12820
21	639.18	13340
22	761.12	14280
23	645.34	13220
24	699.30	13640
25	693.07	13530
26	657.61	12720
27	653.36	12650
28	724.50	13550
29	604.44	11990
30	642.03	12580
31	643.15	12420
32	584.14	12100
33	572.86	11350
34	628.64	12280
35	627.43	11970
36	632.72	11970
37	578.14	11510
38	616.00	11890
39	596.40	11990
40	599.90	12100
Average	643.5 ± 7.26	Average 12506 ± 85.91
Standard deviation	68.06	Standard deviation 805

ounces of buttermilk with 33 grams of barium sulphate. In Table III are reproduced the successive determinations of capacity and the planimeter records of area. Fig. 4 gives the same information in graphic form.

THE REPRESENTATION OF GASTRIC BEHAVIOR

The plotting of calculated volume and measured area of shadow upon millilog paper permits direct comparison of the two resulting curves. That for volume is given both in original computations and in smoothed form. It is apparent that both curves tell the same story. The volume curve has however more marked fluctuations than the curve of area. Naturally the fluctuations of both curves correspond accurately with each other in time relationship. The gastric volume falls for forty seconds after swallowing the meal. This is probably due to the loss of fluid through a patent pylorus. Then with closure of the pylorus the volume rises owing to outpouring of gastric juice. At the end of two minutes an adjustment occurs between flow of juice and rhythmic opening and closing of pylorus so that the volume falls rapidly at first and then progressively more slowly until, toward the end of the experiment the reduction in volume becomes very gradual. These interpretations are based upon other experiments made by Doctor Todd, Miss Kuenzel and others of our staff upon gastric behavior. For further elucidation and explanation the reader must refer to other studies from this laboratory. It is apparent that the computation of volume by Simpson's rule gives a more vivid representation than simple shadow area of the details of gastric behavior. It does not actually bring any new inferences into evidence.

The first striking result of the experiment is the singular similarity in average volume of the stomach under investigation to the average volume of our dead stomachs 2-10 inclusive. This is of course not the average capacity after a hearty meal. What volume the stomach attains after a large meal is a problem for later investigation.

Another result probably of considerable significance is the rhythmic fluctuation in capacity as the stomach empties. This is very clearly shown on the smoothed graph.

The third point to which attention should be drawn is the comparative inadequacy of the first five roentgenograms since barium does not fully canalize the stomach until the sixth, namely two and one-third minutes after swallowing the meal.

It is of course obvious that this method of computation is open to objection. The stomach will not be a perfect tube. The peristaltic waves, in their passage detract somewhat from the reliability of the roentgenographic record. The exact distance of stomach from target is not known, nor is the obliquity of the stomach accurately registered. Valid as all these objections are, their total effect is but a small fraction of the disturbance in gastric behavior caused by the introduction of tube and of air or water.

One must make the reservation that the method here described will give much less reliable results in hypotonic stomachs which hang down in the abdominal cavity as flattened bags.

The probable usefulness of the mathematical method is evident in our routine laboratory studies and may be illustrated in a few sentences. Determination of area as apparent from the graph is subject to modification by irregularities of outline which have less effect upon the record of volume. The latter therefore shows more clearly a rhythmicity of pattern which appears to be characteristic of all our studies. Secondly, by the courtesy of my colleagues I am able to give in Table IV a brief record of other parallel studies made by the application of Simpson's rule in the manner which I have described in this article and illustrated in Table III. Studies in our laboratory have very consistently pointed to a slightly smaller gastric volume after five ounces of water than after five ounces of milk and to a considerably larger gastric volume than that characteristic of milk when the same quantity of buttermilk is administered. Lactic acid results have proved somewhat perplexing and are still being investigated. Very many serial studies indicate that the size and rhythmicity of gastric volume are produced by the interplay of gastric secretion and rhythm of pyloric opening and closing. What apparently erratic influence is its work in our lactic acid studies has not yet been determined.

TABLE IV

COMPARISON OF AVERAGE GASTRIC VOLUME (CC) IN EIGHT SERIAL ROENTGENOGASTRIC STUDIES SHOWING INFLUENCE OF DIFFERENT STIMULI

		MILK	BUTTER MILK	WATER	LACTIC ACID
COV	32	286		254	
TMN	30	248	400		
WFM	31			470	418*
WAS	21		645		510*

*The lactic acid results are so conflicting that some interfering cause is indicated. This is now being investigated.

In conclusion I desire to state that this work was undertaken during the tenure of a Grille Scholarship in the Anatomical Laboratory and I acknowledge my indebtedness to Doctor Wingate Todd for his help in preparing this manuscript for publication.

SUMMARY

- 1 Current estimates of gastric volume suffer from defective technique inasmuch as gastric behavior is influenced by the introduction of unusual foreign substances into the stomach and conditions into the experiment.
- 2 To obviate these difficulties of technique a method is here presented which requires simply an anteroposterior roentgenogram and a determination both of the obliquity of the stomach and the distance of the Magenblase from the anterior abdominal wall.
- 3 Simpson's rule as applied to the volume of a tube of unknown curvature, provides a fairly reliable method of estimating stomach capacity under the particular conditions of our small meal experiments. It also provides a ready measure of the changing volume as contents become transferred to the small intestine.
- 4 The diminishing gastric volume shows rhythmic fluctuations which find their explanation in other studies.

5 Estimated by this method an illustrative five ounce (150 c c) buttermilk meal in a healthy young adult stomach bespoke an average volume of only 643 c c when measured over the first thirteen minutes after swallowing. Since this is greater than the amount swallowed, the secretion of gastric juice must account for the difference.

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LABORATORY METHODS

MECHANICAL AIDS IN LABORATORY PROCEDURES*

By W. J. ROBINSON, TORONTO, CANADA

THE modern laboratory is becoming increasingly dependent upon its mechanical and technical equipment. Technicians, assistants, and professional staff like the artisan to do justice to themselves must be provided with proper tools. Time and thought spent on this phase of the work brings its reward in an efficient and smooth running routine.

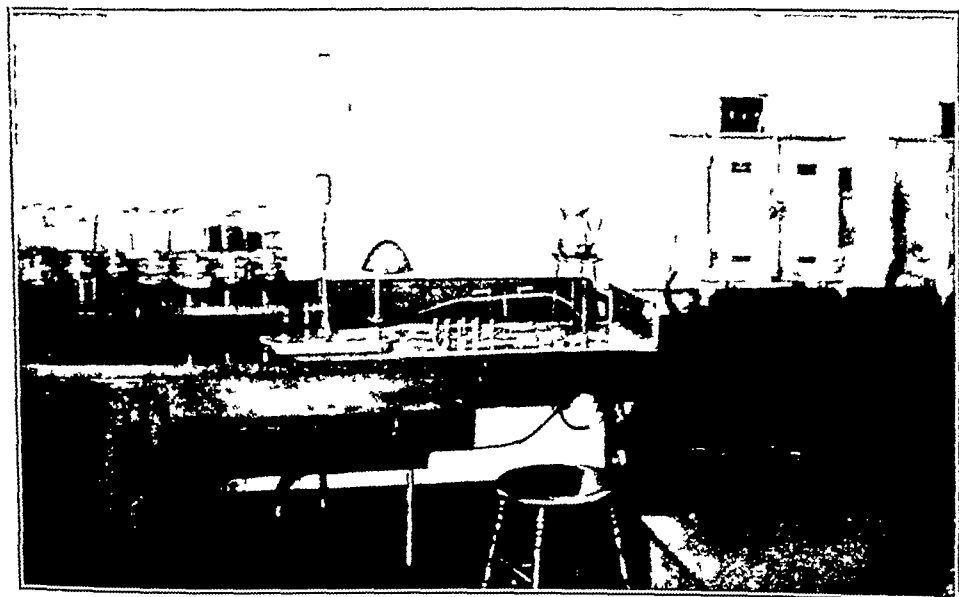


FIG. 1—Shows complete installation of the hot paraffin plate. On the left are the jars of the deparaffinizing fluids and also the staining and dehydrating fluids. On the bench in front of the jars is our special slide rack used for carrying the sections through the various alcohols and dyes.

WARM PLATE FOR PARAFFIN EMBEDDING

Within recent years we have evolved and introduced into our laboratories pieces of apparatus which have proved to be very helpful in the carrying on of the day's work. The first, a warm plate for paraffin embedding has been found to be very useful and to facilitate to a great degree the paraffin embedding of tissue blocks. By our technique the blocks of tissue with respective labels are strung on a fine thread. These are carried through the alcohols into paraffin by the Autotechnicon machine. The string is then placed on

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the warm plate, the blocks cut free with a pair of scissors and embedded in paper boats. This latter procedure is carried out on the warm plate kept at a constant temperature of 70°C . Its construction is simple, consisting as it does of a sheet of copper 18×36 inches with the edges rolled and corners soldered, except at one end where the sheet dips over into the sink. To the bottom of the sheet and 6 inches from the sink end there is soldered a copper box 4 inches square by 3 inches deep. A hole is cut through the copper sheet and a spout inserted to correspond to one corner of the box beneath. This is used to fill it with water. A heating element consisting of an ordinary plug-in electric heater used for hot water boilers is inserted in one side. A screw nut is sweated into the side of the box and the heater screwed in. In a similar

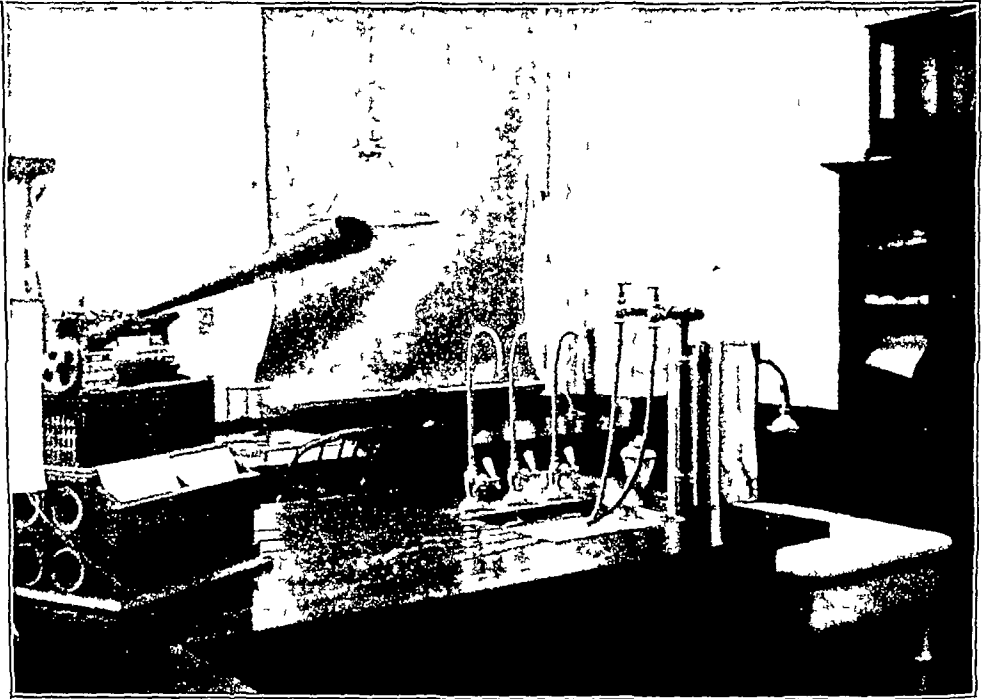


Fig 2—Completely equipped workbench for surgical gross material including dictaphone sink hot and cold water special taps for formalin alcohol and distilled water and at the end of the bench a surgeon's washbasin with knee controlled water supply

manner an electric thermostat is inserted. These are connected to a floor plug and a switch with pilot light wired in the line. The complete installation, including the paraffin oven, sink and staining jars, is shown in Fig 1.

A PRESSURE SYSTEM FOR FORMALIN ALCOHOL AND DISTILLED WATER

Instead of the gravity system with its unsightly row of large bottles overhead we have installed a pressure system with ordinary water taps for distributing the formalin, alcohol, and distilled water as required. The large winchesters containing these fluids are placed in the locker space beneath the workbench. A pressure is piped to the bench and a pressure reducing valve inserted in the line. The winchesters are closed with rubber corks with two glass

tub's running into each. One goes to the bottom, the other just through the cork. The latter is hooked up to the air pressure line, and the former by rubber tubing is connected to a goose-neck tip which drains into the sink, as shown in the illustration (Fig. 2). The taps are of the ordinary quick action lever type. These are stock equipment which may be purchased from any plumber. We have found that an air pressure of three pounds is the most satisfactory, producing a good flow of fluid without much danger of blowing the corks out of the bottles.

MICROTOME KNIFE SHARPENER

The need for such a machine is evidenced by the many new devices reported for this purpose in the literature. The principle involved is not new. Its application for this purpose has worked out very satisfactorily. It consists essentially of two steel rollers 14 inches in length by $3\frac{1}{2}$ inches in diameter with a shaft running through the center. Two six inch wide bands

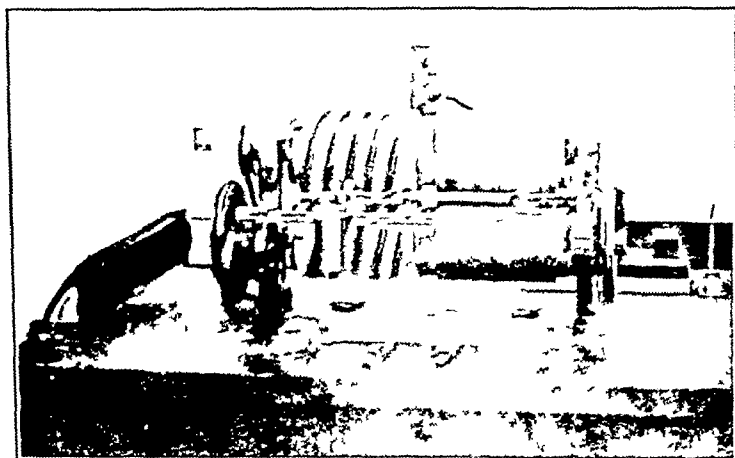


Fig. 3—Front view of microtome knife sharpener showing the smooth leather rollers on the right for grinding and the spiral leather strap on the left for finishing. The large pulley at the left end actuates the cam shaft.

of heavy leather belting are cemented on to each roller. The band on the right end of the roller is built up until a thickness of about $\frac{1}{2}$ inch is attained. The roller is then put in a lathe and the leather very carefully turned down until a thickness of about $\frac{1}{8}$ inch is left. It is smoothed down with emery paper. The leather band on the left end of the roller is cemented on in the same manner until a thickness of about $\frac{1}{4}$ inch is attained. This is turned down in the lathe to $\frac{1}{8}$ inch in thickness. A $\frac{1}{4}$ inch thickness of sponge rubber is then fitted over this and cemented to the underlying leather. Where the edges meet a row of fine tacks are put in to prevent the rubber from curling. Over this are wound three carefully selected pieces of leather strapping $\frac{1}{2}$ inch in width and $\frac{1}{8}$ inch in thickness. These are fastened at each end by drilling a hole into the steel roller, tapping it and inserting a screw. The leather straps are wound around the roller in a spiral fashion. The other roller is prepared in exactly the same manner. The rollers are mounted in brass bearings and supported on a cast iron frame (Fig. 3).

A holder for the knife is shown in position attached to the rocker bar by a thumb-screw in Fig 4. It is constructed of a metal bar $4\frac{3}{4} \times 1\frac{1}{2} \times \frac{1}{2}$ inches with a slit in the center for the screw-bolt to hold it to the rocker bar. On one end is fastened a counter-balance weight and on the other a metal jaw to hold the knife. This jaw fits into the first bar by a pin and socket joint so that it can rotate sideways and allow for an even pressure on the rollers.

The rocker bar is supported at each end in brass bearings and actuated by a cam located underneath at the left end (Fig 3). The cam is rotated at a speed much less than that of the rollers by reduction gears. By this bar with its cam the knife is pressed first against one roller then the other. One turn is required to throw the knife from the one roller to the other. It is then held against the roller for three turns. It is our intention however to put in more gears to reduce the speed of the rocker arm shift so that the knife will be held in contact with the roller for some 12 to 14 turns each.

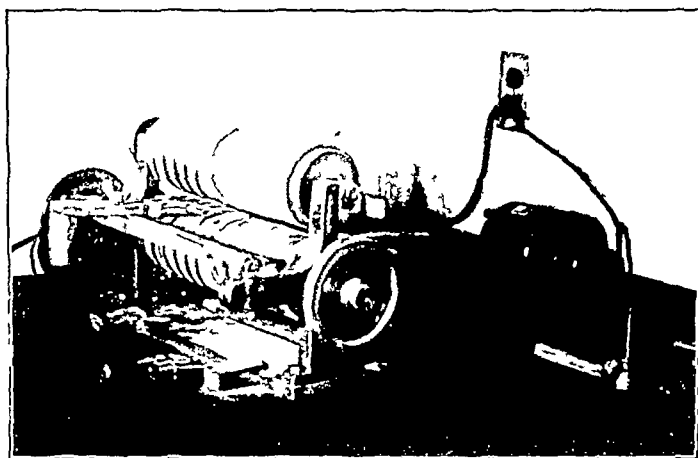


Fig 4.—Side view of microtome knife sharpener showing the knife in position with its edge properly in contact with the finishing roller to correspond to its final bevelled edge.

The rollers are connected up by round leather belting over pulleys. The two rollers are run in opposite directions and away from the edge of the knife. The proper adjustment of the knife holder on the rocker bar is very important. The distance in or out determines the final bevel edge of the knife. Once established this should be kept the same. We found it an advantage to grind all the knives to the same bevel then to leave the knife holder locked in place and just slip the various knives in or out of place by releasing the screw on the jaw at the end which clamps the knife. The pressure of the knife on the rollers is adjusted by a spring tension on the two arms attached to the rocker bar which ride over the cam. This adjustment we have found to be very important and will probably lead to our making some further changes in design to more adequately control it. The machine in principle seems to be sound. We do feel however that many improvements can be made in the way of finer adjustments. The character of the leather strap used is important. In the case of the spiral straps these must be kept clean and occasionally oiled. The leather rollers on the right side must be

turned down as true and smooth is possible. They are oiled and a fine grade of aloxite powder rubbed in to leave proper grinding surface. The machine is run by a small $\frac{1}{10}$ horse power electric motor.

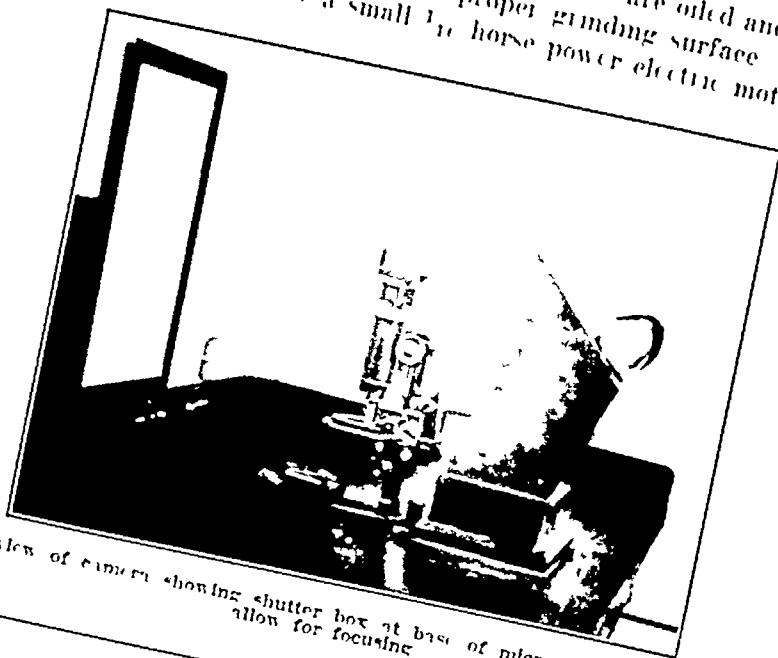


Fig. 5—Side view of camera showing shutter box at base of microscope pushed forward to allow for focusing

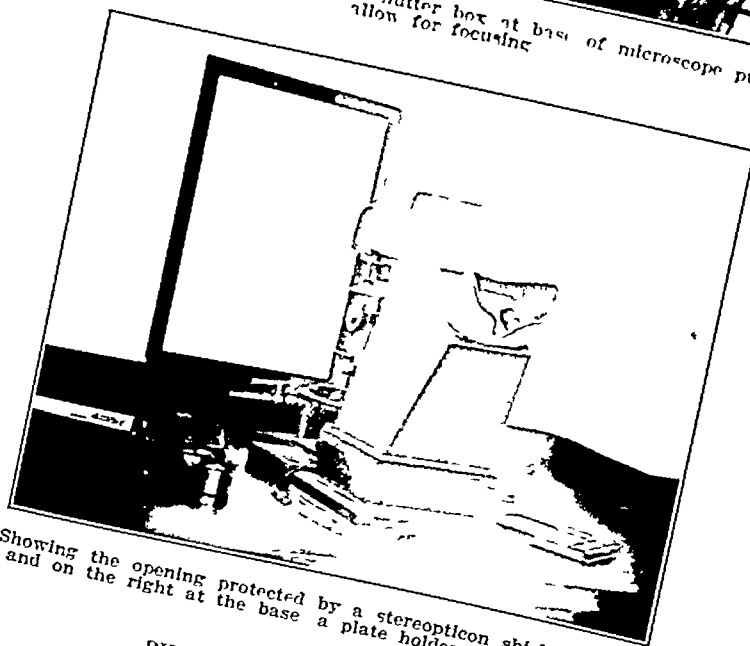


Fig. 6—Showing the opening protected by a stereopticon shield for viewing the image while focusing and on the right at the base a plate holder partly inserted into position for exposure.

PHOTOMICROPATHIC CAMERA

This was designed to meet the need for a camera which would be simple to handle and ready at hand. It is securely mounted on one's workbench with microscope and light permanently placed. Interesting or important sections can be photographed at once no time being lost in going to another department or in making camera adjustments. Its limitations in magnifica-

tion are broad enough to allow for 90 per cent or more of the work. The lowest magnification we have found satisfactory for general purposes is $\times 48$. If lower is needed the camera can be set a little higher off the bench to allow for a greater extension of the microscope barrel. The upper limit of magnification is about $\times 800$, although we have taken pictures up to $\times 1800$. These latter are difficult to make and usually not altogether satisfactory.

The camera consists essentially of a light wooden box, light-proof, and built as shown in the illustrations (Figs 5 and 6). A wooden spout projects forward 7 inches. It is $2\frac{3}{4}$ inches square at the camera end and reduces to $2\frac{1}{8}$ inches square at the distal end. A piece of $1\frac{1}{2}$ inch thin brass piping is inserted in a hole on the under surface of the spout at its outer end and allowed to project down $\frac{1}{4}$ inch. This provides an opening for the microscope on which should be placed a circular metal light-trap. The distal end has an opening cut at an angle of 45° which is covered by a wooden block

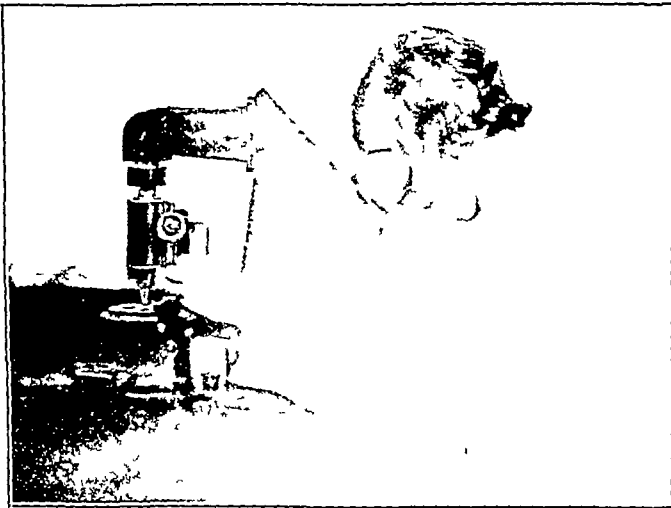


Fig 7—The camera in use showing its ease of handling and accessibility of focusing parts

rounded off on its upper surface and countersunk on its under surface. It is hinged to the spout. To the under surface of this block is attached a $\frac{3}{4}$ inch right angle prism set so that the rays of light coming from the microscope are deflected at a right angle through the spout to a surface mirror in the top of the camera box. The camera is constructed so that a 4×5 plate holder will slip into its base. The plate holder is held up in position by a wooden plate $4\frac{3}{4} \times 6\frac{1}{2}$ inches with three coiled springs under it. The upper surface is painted white. This serves as a focusing screen. It is cut down a sixteenth of an inch to give it the same level as a plate in its holder. Fig 6 shows a plate holder on the right side of the camera partly inserted into its proper position. The camera is set on a board $7 \times 8\frac{1}{4} \times \frac{3}{4}$ inches in thickness. If a taller microscope is used one or more boards may be placed under the camera and screwed down to the table until the proper height is attained for the free movement of the microscope barrel within the limits of magnification required.

The main body of the camera is constructed with bevels to reduce its bulkiness. Its base (outside measurement) is $5\frac{1}{2} \times 6\frac{1}{4}$ inches and its height to the apex of the camera in front is 13 inches. The top is cut at an angle of 45° and toward the front an opening is left in it $4\frac{1}{2} \times 4\frac{1}{2}$ inches with countersunk edges. Over this is placed a lid with overhanging edges so as to exclude light and to the under surface of this is attached a surface mirror measuring $11\frac{1}{2} \times 21\frac{1}{2}$ inches. This mirror reflects the image from the prism down onto the screen board at the base of the camera. The length of the spout and the height of the camera is constructed so as to give a projection length from the ocular of the microscope to the plate in its holder of 500 mm. This is double the normal projection length of a microscope and therefore doubles its magnification. The image is viewed through an opening in

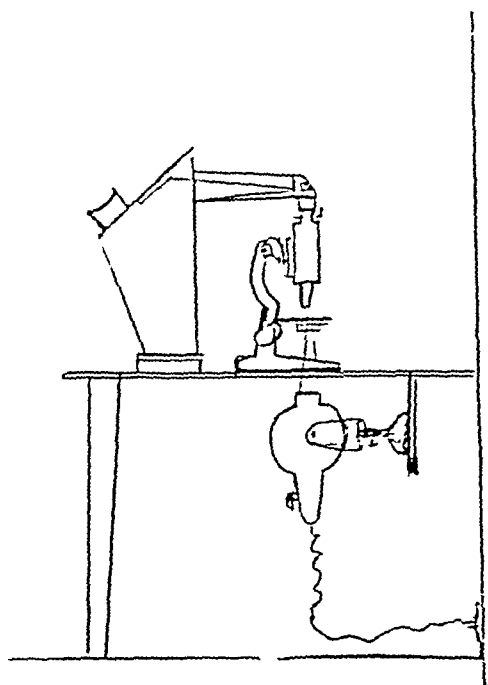


Fig. 8—Diagrammatic drawing of camera and microscope in position on top of the bench with the Punktlicht below.

the top of the camera toward the back which is protected by an ordinary stereopticon shield as illustrated in Fig. 6. When the focus has been accurately adjusted the slide is dropped down over the viewing holes and the plate holder inserted.

As an illuminant we use a Zeiss Punktlicht. This is mounted beneath the bench (Fig. 8) on a crossbar so that it can be moved sideways as well as back and forth in order to center it beneath the substage condenser. A hole $1\frac{1}{2}$ inches in diameter is bored in the bench top to allow the light through. Once it is properly placed it will not have to be touched again. To focus it the substage condenser of the microscope should be removed. The light is then adjusted to a sharp focus on the object slide. The substage condenser is then put back into place and adjusted with its rack and pinion to

bring out the clearest delineation of the image. For very low power work we remove the front lens of the substage condenser. To control the exposure a small inverted wooden tray slides over the legs of the microscope stand and in it is bored a hole to come exactly over the beam of light. Into this hole is screwed an ordinary camera shutter of the varying speed type. This is controlled with a plunger type of release, Fig. 5. To focus the section the tray with shutter is slid aside. When the focusing is completed it is shoved back into place and the shutter used. Whatever colored screen is required is simply placed on top of the shutter as illustrated in Fig. 6.

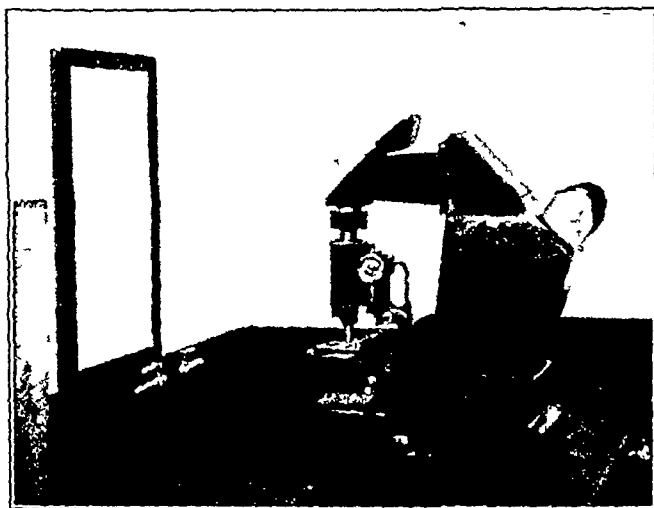


Fig. 9.—The camera being used as a projector. The image is thrown onto the screen in front by merely slipping an extra mirror into the end of the spout over the microscope.

One can see from Fig. 7, how readily accessible are all the controls on the microscope and how easy it is to focus the image and make the exposure. While the camera is constructed to use 4×5 inch plates we put in kits and use $3\frac{1}{4} \times 4\frac{1}{4}$ inch plates. Lantern slides are then made by direct contact printing. If larger prints are desired enlargements may be made from the original negative.

The camera can be put to another use namely, a projector for demonstrating slides to small groups. This is done by merely lifting the hinged wooden block on the front of the spout and inserting a small mirror into a groove cut at an angle of 45° as shown in Fig. 9. The image from the microscope is then thrown forward onto a screen placed on the wall in front of the camera.

A METHOD FOR THE STANDARDIZATION OF COLLOIDAL GOLD SOLS IN THE LANGE TEST*

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THE precipitation of colloidal gold by cerebrospinal fluid was first investigated by Carl Lange¹. He found that colloidal gold was not precipitated by cerebrospinal fluid obtained from normal individuals, but that those from individuals suffering from certain affections of the central nervous system gave definite precipitation reactions. He observed that these reactions invariably occurred with cerebrospinal fluids obtained from patients with syphilis of the central nervous system and considered that the colloidal gold test was of great value in the diagnosis of such conditions. These observations of Lange have been subsequently confirmed by various observers.

In 1914 Miller and Levy² emphasized the fact that the precipitation of colloidal gold by spinal fluids from patients with tabes differed in a fundamental manner from those obtained with spinal fluids from paralytics. Miller, Brush, Hammers and Felton³ confirmed these observations. They concluded that the reactions are characteristic with spinal fluids of general paralytics but not with those in tabes nor in cerebrospinal syphilis. They also observed that the typical paralytic curve was obtained occasionally with spinal fluids obtained from syphilitic patients showing no evidence of dementia.

Mechanism of the Gold Sol Reaction With Cerebrospinal Fluid—The mechanism of the reaction between the colloidal gold sol and the spinal fluid has been the subject of many investigations. Lange¹ assumed that the reactions were due to the presence in the spinal fluid of specific proteins. Weston⁴ found that the gold precipitating substance is not the Wassermann producing substance since separation of the two could be effected. He suggested that the active precipitating substance in the spinal fluid is a globulin, since it is precipitated from solution by ammonium sulphate, and is destroyed by heat. It is necessary to note that Weston did not consider an increase in globulin alone as indicative of syphilis because such an increase is found in the spinal fluid in many conditions other than syphilis.

Felton³ considered that the various types of reaction were due to the antagonistic precipitating relations of albumin and globulin, the albumin exerting an inhibitory action and the globulin a precipitating action. The results obtained by Fischer⁵ indicate that all four of the globulin fractions (fibrinogen plus fibrinoglobulin, euglobulin, pseudoglobulin I and pseudoglobulin II), possess a flocculating effect upon the colloid gold sol. These different globulin fractions in similar concentrations exert flocculating effects which vary in degree. Both the first and second albumin fractions fail to

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cause flocculation of the colloidal gold but have a protective effect tending to prevent such flocculation. The amount of flocculation is proportional to the concentration of the globulin solution.

Spat's⁷ theory that in syphilitic involvement of the central nervous system the flocculation of the gold depends upon the syphilitic antibodies was disproved by these experiments. Lange's⁸ and Eicke's⁹ theory that from the result of the colloidal gold reaction the strength of the Wassermann reaction may be predicted is valid only in certain cases. In some cases of cerebrospinal syphilis, the Wassermann reaction of the spinal fluid is negative in spite of considerable flocculation of the gold. The expulsion into the spinal fluid of the syphilitic reagent from the infected central nervous system does not always run parallel with the exudation of the globulin.

Independently of Fischer, Ciniackshank¹⁰ made a detailed investigation into the value and mechanism of the colloidal gold test. He confirmed the conclusions of Weston that the active substance in the spinal fluid in general paresis is not dialysable and that it resides in the globulin fraction of the protein but stated that it is not affected by heating to the coagulable point of the protein. He observed that the various types of the syphilitic reactions could be simulated by mixtures of globulin and albumin, the globulin acting as the precipitating agent and the albumin as the protective agent. He therefore concluded that the syphilitic reactions are in part due to the presence in the spinal fluid of albumin sufficient in quantity to partially obscure the precipitating effects of the globulin, and in part also to a specific alteration in the physical state of the globulin which is associated with a positive electrical charge.

It appears then that the type of reaction obtained with any given abnormal spinal fluid depends upon the balance between the albumin and the globulin content of the fluid. When globulin is present along with little albumin, the flocculation of the gold begins in the lowest dilutions of the spinal fluid and tends to disappear in regular gradations in the higher dilutions after the precipitating effect of the globulin has been dispersed by the dilution. This type of spinal fluid is rather constant in general paresis, and occurs with considerable frequency in multiple sclerosis. It gives the so-called "paretic type" colloidal gold curve in the test.

In spinal fluids containing a similar amount of globulin, but a greater amount of albumin than occurs regularly in spinal fluids of the paretic type, incomplete precipitation occurs in the lower dilutions of the fluid. The amount of precipitation increases with the dilution for a time to a peak after which further dilution brings about a regular decline in the amount of flocculation. This gives what is known as the "tabetic" type of gold sol curve. With such fluids the protective effect of the albumin must be dispersed by dilution before the precipitating property of the globulin brings about its full effect. The increasing precipitation with dilution is due to the elimination of the inhibiting effects of the albumin, while globulin concentrations remain active. Upon further dilution, the precipitating effect of the globulin is weakened so that a gradual decline of the curve occurs.

When both the albumin and the globulin contents of the spinal fluid are greatly increased over the amounts present in the above types the same phenomena are observed as occur in the "tubercle type" of reaction. Here however the inhibiting effects of the albumin will be carried through a wider range of dilutions and after the albumin effect disappears, there yet remains sufficient globulin concentrations in the higher dilutions to bring about complete precipitation of the gold. The highest dilution used routinely in the test (1:5120) may cause complete precipitation of the gold used. If still higher dilutions are made however a point is reached where the precipitating effect of the globulin declines rather abruptly. This type of fluid is found usually in acute meningitis. Chronic forms of meningitis may give rise to it but more frequently such fluids tend to approach more nearly the reactions of the "tubercle type".

Methods Herebefore Advocated for Adjusting Gold Sols for a Standard Reaction—One of the difficulties met with in applying the colloidal gold test has been that of standardizing the sensitiveness of the gold sol to be used from time to time in the tests. Adams and his associates¹¹ investigated the P_H readings of sols and found that a P_H of 5.2 yielded sols of satisfactory and fairly constant sensitiveness. In this method a mixture of 8 c.c. of the sol and 1 c.c. of a 5 per cent sodium chloride solution in triple distilled water is allowed to stand for twenty-four hours. Upon the precipitation of the gold 2.25 c.c. of the clear supernatant liquid (representing 2 c.c. of the original gold solution) is titrated with $N/500$ hydrochloric acid to the maximum yellow with brom cresol-purple i.e., P_H 5.2. According to this titration the bulk of the solution is then adjusted and the P_H reading checked.

The hydrogen-ion reading may be made on the gold sol without precipitating the gold if the dye is added directly to the gold solution and a tube of gold sol without dye used in front of the color standard as a color compensator when bromthymol blue is used as the indicator. If the precipitation method is preferable the whole determination may be hastened by centrifugalizing after the addition of the sodium chloride solution so as to obtain the clear liquid in a few minutes.

The older method of testing the sensitiveness of the gold sol by the use of 1 per cent sodium chloride solution is useful as a preliminary test. The addition of 0.4 c.c. of the sodium chloride to 5 c.c. of the gold sol should produce no precipitation as evidenced by a color change, while the addition of 1.7 c.c. of the 1 per cent solution of sodium chloride should produce complete precipitation of the gold. We have found that more information may be gained from this test if the amounts of sodium chloride solution added are so chosen as to bring out partial precipitation of the gold. It has been found that the above tests supplemented by the addition of a third tube in which 1 c.c. of the sodium chloride solution is mixed with 5 c.c. of the gold proves more useful as a preliminary indication of the sensitiveness of the gold. A gold sol of the proper sensitiveness will produce a reading in this tube of not greater than "2" after one hour at room temperature. The attempt to use this reaction in determining the amount of acid or alkali to be added to a gold sol for the proper adjustment of its sensitiveness has not met

with success, chiefly because the response to precipitation with this amount of sodium chloride is not delicate enough after the additions of graduated increments of very dilute acid or alkali throughout a series of tubes to bring out color changes proportionate to the amounts of acid or alkali added to the gold in the various tubes

Considerable experience with the method of adjusting the sensitiveness of the gold sols by means of the adjustment of the hydrogen ion reaction demonstrated to us that there was another factor aside from the reaction which influenced the response of the gold to precipitation in the spinal fluid test. In the preparation of the colloidal gold we have used the following technique. 10 cc of a 1 per cent gold chloride solution (Merck's Blue Label gold chloride in double distilled water) is added to 1000 cc of double distilled water which has just reached a temperature of 60° C. The heating is done over a Bunsen burner with the water in a 2-liter Erlenmeyer flask supported on a tripod covered with a wire gauze. Immediately after the addition of the gold chloride solution, 7 cc of a 2 per cent potassium carbonate solution is added and the heating continued until the temperature reaches 90° C. The flask is removed and placed on a towel folded so that several thicknesses permit handling with adequate protection from the heat. Formaldehyde, 1 per cent solution, is now added while the flask is being shaken thoroughly. Small additions are thus made until the appearance of a slight pink tinge appears throughout the liquid. Usually about 10 cc of the formaldehyde solution are required. The shaking is now stopped and the reaction goes on rapidly to completion. This method has yielded satisfactory clear red gold sols with a high degree of consistency. Failures occur occasionally but these can usually be traced to some variation from the standard technique, at times even to such a small consideration as the fitting of a new cork stopper to some part of the glass apparatus used in the preparation of the distilled water. Usable golds must have little or no turbidity when viewed by reflected sunlight in thick layers. The amount of this turbidity though slight varies with the different lots of gold sol prepared from time to time. The appearance of this turbidity on reflected light is an expression of the amount of relatively large particles of gold in the sol. When the amount of large particles is sufficient to give a slight violet tinge to the sol when viewed by transmitted light the sol is unfit for use.

Given two usable gold sols of like P_H concentration but differing slightly in the amount of this turbidity by reflected light it will be found that the one showing the more turbidity will be the more sensitive when used in the test with spinal fluid. This then has been found to constitute a second factor in the standardization of gold sols. It may be expressed briefly as follows: the sensitiveness of a gold sol in the reaction with spinal fluid varies directly with the size of the colloidal particles, when the P_H is constant. It follows, since acid reactions tend to increase the sensitiveness, and alkaline ones to decrease the sensitiveness, that the P_H concentrations which must be used to adjust gold sols differing slightly in turbidities to a standard sensitiveness will vary somewhat. Very clear gold sols when adjusted to this standard will show P_H readings of from 6.0 to 6.4, while the very slightly turbid ones when ad-

justed to this standard will show readings of from P_H 6.5 to 6.8. These two factors—the size of the colloidal particles and the P_H concentration of the gold sols—are inseparable and must be considered of the greatest importance, for on them depends the sensitiveness of the gold. Crickshank¹⁰ and other investigators have pointed out that acid sols are too sensitive and alkaline ones are insensitive. Our observations have demonstrated that slightly turbid gold sols require greater amounts of alkali and higher P_H values than do very clear golds. Because of this variation of the P_H values with the size of the particles, a method of adjustment of the reaction on the strength of a test depending only on the hydrogen-ion reaction is unsatisfactory. Both factors must be considered, and can best be taken into account by the method to be presented herein.

Preserved Spinal Fluid Essential as a Standard—In the method described in this paper a preserved spinal fluid is essential. In the course of a large experience with the colloidal gold test it occurred to us, that the most certain method of taking into consideration all the factors concerned in standardizing the sensitiveness of different gold sols in this test, had as its basis the discovery of a means to maintain constancy in the reacting strength of a given spinal fluid over a period of time. If this could be accomplished each new gold sol could be used in an actual test against such a spinal fluid, and by proper additions of acid or alkali different gold sols could be adjusted to give the desired reaction with this spinal fluid. This would mean that different samples could be made to react in a standard manner with this one spinal fluid. It was soon learned that gold sols adjusted to a constant reaction with a given positive spinal fluid of the "paretic type" could be depended upon to react in a constant manner with normal spinal fluids and with the different types of abnormal fluids found in the routine application of the test.

Various methods of preserving spinal fluids were tried in an attempt to find a means of maintaining a constant reacting value of a given fluid over a considerable length of time. It was found that the addition of glycerin in 50 per cent concentration offered the best possibilities in this regard. Spinal fluids showing typical "paretic type" reactions were selected from week to week over a period of a year. To each was added an equal volume of glycerin. After thorough mixing the glycerinated specimens were stored in the refrigerator. Records were kept of the original reactions in the colloidal gold test, and from time to time the test was repeated. Of 48 specimens on which sufficient data have been recorded the results have been as follows: all gave the

TABLE I

SHOWING MANNER IN WHICH PRESERVED SPINAL FLUIDS RESPOND IN THE COLLOIDAL GOLD TEST OVER A PERIOD

Spinal fluid I,	1/28/25.....555554321	Spinal fluid II,	3/13/25.....555432100
	3/13/25.....555554321		4/27/25.....555432100
	4/27/25.....555554321		5/12/25.....555432100
	5/12/25.....344554321		6/16/25.....455543200
Spinal fluid III,	4/27/25.....555554321	Spinal fluid IV,	4/27/25.....555554321
	5/12/25.....555554321		5/12/25.....555543210
	6/16/25.....555554321		6/16/25.....555543210
	7/17/25.....3344554320		7/17/25.....4455543211

original readings after four weeks, 47 after five weeks, 44 after six weeks, 38 after seven weeks, 35 after eight weeks, 31 after nine weeks, 22 after ten weeks, 8 after eleven weeks, and none after twelve weeks

It will be noted that when deterioration begins it is evidenced by changes in the reactions with the lower dilutions of the fluids. This has been a constant finding in all of our preserved spinal fluids yielding initial reactions of the "paretic type." Partial instead of complete precipitation in the lower dilutions marks the beginning of deterioration of the preserved specimens. More important is the fact that the higher dilutions in which originally partial precipitation occurred ("4" and "3" readings), continue to give their initial readings even after the altered readings appear in the lower dilutions. These dilutions showing the initial partial precipitation are the dilutions of choice in adjusting different gold sols to a constant reaction, so that as above indicated, a preserved spinal fluid may continue to be of use for purposes of standardizing gold sols, even after it begins to show altered reactions in the lower dilutions. Their use for this purpose after the initial reading no longer appears is not recommended.

The Choice of a Spinal Fluid for Use as a Standard—Given a method of preserving a constant reacting value of a spinal fluid over a time sufficiently long to make its use as a standard practical, an important consideration arises in regard to the choice of the spinal fluid to be used as the standard. We wish to make it clear at the start that such choice must in a sense be empirical since a uniform reacting strength in the colloidal gold reaction is not a property of paretic spinal fluids. It is true, however, that the reacting strengths of paretic spinal fluids are more constant than that of abnormal spinal fluids from any other clinical conditions. At most the variations in the number of dilutions giving complete precipitation vary not more than two tubes on one side or the other of a hypothetical standard which yields complete precipitations in the first four dilutions (1:10, 1:20, 1:40, 1:80), and the usual variation is not more than one tube dilution over or under these dilutions. When one couples these considerations with that further consideration that the zone of sensitiveness of a gold sol for practical results in this test is confined within narrow limits, an empirical choice of the paretic spinal fluid to be used as a standard is made easier and without a great chance for variations between standards chosen by different experimental workers.

Of greater importance than the matter of the choice of a paretic spinal fluid are the properties of the sample of gold chosen as the normally reacting gold to be used in obtaining a reaction with the preserved spinal fluid. Our aim has been to make the routine colloidal gold tests as sensitive as possible by choosing a degree of sensitiveness for the gold just short of that which would give slight precipitation in the middle zone of the dilutions in the routine test with normal blood-free spinal fluids. Arrival at such a standard can be made only after extensive trial with normal spinal fluids in the regular manner of testing. Preliminary to such trials with normal spinal fluids the gold sol should be shown to have the following properties. It should be clear and show no color change upon the addition to 5 c.c. of the gold sol of 0.4 c.c. of a 1 per cent sodium chloride solution. The addition of 1.7 c.c. of a

1 per cent sodium chloride solution to 5 c.c. of the gold sol should cause complete precipitation upon standing overnight. The addition of 1 c.c. of a 1 per cent solution of sodium chloride to 5 c.c. of the gold sol should produce upon standing overnight a color change adjudged to be midway between a "1" and a "2" reading. With spinal fluid from a known parietic, the gold sol should give a parietic type curve. The satisfactory outcome of these preliminary tests will indicate that the sample is suitable for application in the tests with a series of normal blood free spinal fluids which will determine very definitely the desired degree of sensitiveness. Fifteen or twenty such samples of spinal fluid will suffice. Divide the sample of gold sol into three portions each of which should be of sufficient bulk to do a set of tests with the chosen number of samples in the regular manner. First, the unaltered sample is used with all of the negative spinal fluids. If upon standing overnight no middle zone precipitation occurs, the gold sol may be either of the proper sensitiveness or it may be slightly insensitive. In this case the two remaining portions of the gold sol are used after addition to the one of 0.1 c.c. of N/5 HCl per liter, and to the other of 0.2 c.c. of N/5 HCl per liter. Routine tests with the chosen samples of negative spinal fluid are again set up with the sample of gold sol containing 0.1 c.c. per liter of N/5 HCl. Should middle zone precipitation appear, it indicates that the unaltered gold sol is of the proper degree of sensitiveness. Should none appear, the tests are made with the sample of gold sol to which 0.2 c.c. of N/5 HCl was added. The degree of sensitiveness which is represented by that just above where the first consistent middle zone precipitation appears in such tests is the one desired.

If the first series of tests with the unaltered gold sol should show middle zone precipitation, then the same procedures are employed using N/5 NaOH instead of the HCl.

The degree of sensitiveness which we employ then may be defined as that which is removed by not more than 0.1 c.c. of N/5 NaOH per liter from the sensitiveness which gives partial mid zone precipitation with normal spinal fluids. This gives a very definite determination of the sensitiveness of the gold sol to be used in obtaining readings with the parietic spinal fluid to be used as standard, but it is obviously cumbersome and applicable only in laboratories where abundant clinical material is available for study. We have more recently been able to define the degree of sensitiveness in terms more definite by means of a standard solution of globulin (see next paper). Once this determination is made, and a reading obtained by the use of the gold sol with a parietic spinal fluid, such reading will enable one to adjust very simply subsequently prepared samples of colloidal gold to it. By the choice and preservation of positive spinal fluids from time to time the standard may be maintained indefinitely according to the plan outlined below.

NEW METHOD FOR THE ADJUSTMENT OF GOLD SOLS

Solutions required

- 1 Sodium chloride solution, 0.4 per cent
- 2 Hydrochloric acid, N/5 and N/1000
- 3 Sodium hydroxide, N/5 and N/1000

- 4 Preserved spinal fluid This spinal fluid is preserved by the addition of an equal volume of glycerin. As shown above spinal fluids preserved in this manner will yield constant readings over a period of several months. It is the practice in this laboratory to use no standard preserved spinal fluids more than a month old.

Technic of the Method—The gold sol is prepared and should be allowed to stand for at least two hours before attempting to standardize its sensitiveness. The preserved spinal fluid giving initially a positive paretic type curve is then made up in the dilution at which the first partial precipitation of the gold, that is a "4" reading, is known to occur. This dilution is made in 0.4 per cent sodium chloride solution. One c.c. of the diluted spinal fluid is placed in a test tube and 5 c.c. of the gold sol is added. A "4" reading should result if the gold sol is of the proper sensitiveness. The reading is made after the tube has been allowed to stand for eighteen hours. If this reading does not correspond to the desired "4" reaction the variation may occur on either side of that desired. If the gold sol is too sensitive complete precipitation will result, i.e. a "5" reading. If the gold sol is not sensitive enough, less than a "4" reading will appear. Should the gold sol appear too sensitive, alkali must be added, if not sensitive enough acid must be used in the adjustment. The following would represent the titration scheme in case the gold sol appears insensitive.

TUBE NO	1	2	3	4	5	6	7	8	9	10	11
N/1000 HCl	0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
Gold sol	5	5	5	5	5	5	5	5	5	5	5
Sp. fld 1 (1:320)	1	1	1	1	1	1	1	1	1	1	1
Readings	2	3	3	4	4	5	5	5	5	5	5

In this instance it is noted that the partial reaction desired (the "4" reading) occurs first when 0.15 c.c. of N/1000 HCl is added to 5 c.c. of the gold sol. From this a simple calculation serves to show that 0.15 c.c. of N/5 HCl is required per liter for the proper adjustment of the gold sol.

If the gold sol had appeared too sensitive, a similar titration substituting N/1000 NaOH for the HCl would have been used. This reading may be made after eighteen hours at room temperature.

After the main sample of the gold sol has been adjusted by adding the calculated amount of N/5 HCl or NaOH, a 5 c.c. sample is tested with 1 c.c. of the diluted spinal fluid standard. A "4" reading should result, and the gold sol is ready for use in the routine tests.

These routine tests are controlled by setting up the regular test with the preserved spinal fluid as a positive control. In using this it must be remembered that 0.4 c.c. of the glycerinated sample and 1.6 c.c. of 0.4 per cent sodium chloride solution are placed in the first tube instead of the usual 0.2 c.c. of spinal fluid and 1.8 c.c. of the saline since the preserved spinal fluid has already been diluted with an equal part of glycerin.

Advantages of the Method and Practical Results—This method for adjusting the reaction of gold sols is far more accurate than any heretofore suggested. The ease with which the calculations are made adds accuracy and simplicity. The time consumed in performing the test is comparatively short.

for it has been our experience that the first two titrations usually suffice if the instructions for preparing the gold are followed. Consequently, the third titration becomes necessary only in special cases. The method has been in use in this laboratory during the last five years in preparing the gold sols for over 18,000 tests and has never failed to yield satisfactory results.

DISCUSSION

The absolute necessity of standard gold sols in adjudging the results of treatment in cerebrospinal syphilis is self-evident. The lack of dependable methods for the adjustment of the reactions of the gold sol and the absence of uniformity in the methods in use make it fallacious to compare colloidal gold tests performed in different laboratories. One can hardly get the correct idea of the facts clinically from such comparisons. Nor can a true idea of the results of treatment in the cerebrospinal syphilis be obtained even if all the tests performed on a particular patient are carried out in the same laboratory because of the inability of previous tests to adjust gold sols so that they will be constantly uniform.

We believe that the method herein described will correct these conditions and will assist the clinician to adjudge properly tests performed in different laboratories and on the same patient to show the progress of the treatment.

SUMMARY

Colloidal gold reactions of the paretic and the tabetic type may be regarded as of diagnostic value while the meningitic reactions are less reliable.

Reactions of the cerebrospinal fluid with colloidal gold appear to be due in part to the presence of albumin sufficient in quantity to partially obscure the precipitating effect of globulin and in part to a specific alteration in the physical state of the globulin which is associated with a positive electrical charge.

Methods of adjusting gold sols alone dependent of the P_H value of the gold are not satisfactory.

A preserved standard spinal fluid is essential.

A satisfactory method for adjusting the sensitiveness of gold sols must take into account the fact that the sensitiveness of gold sols depends on two factors, first, the size of the colloidal particles and second the P_H value of the gold sol.

Turbid gold sols require greater amounts of alkali and higher P_H values than do clear golds.

The method herein described is accurate, simple, non-time consuming and gives uniform results. Consequently, it makes possible the proper adjudging of tests done in various laboratories and on the same patient at different times.

Addendum—Glassware should be left overnight in cleaning solution (sulphuric acid and potassium dichromate) and then rinsed three times in tap water and three times in distilled water. Clean glassware is essential. Gold

sol contains one part gold in 10,000 parts of distilled water so that a minute trace of acid attains a high value when compared in molecular magnitude to the gold

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A METHOD OF STANDARDIZING COLLOIDAL GOLD SOLS BY UTILIZING A STANDARD SOLUTION OF GLOBULIN*

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THE method of adjusting colloidal gold sols previously described† is quite satisfactory where spinal fluids of paretics may be obtained from time to time. Since these are not readily available in laboratories of small hospitals or in public health and biologic laboratories, we undertook the preparation of a standard solution of globulin to be used instead of such spinal fluids. A good grade of globulin available commercially was found to serve this purpose admirably‡ and the stock solutions of it prepared as described below have been found to give identical results over periods of from five to six months. The different samples of this product purchased from time to time have been found to be very consistent in precipitating colloidal gold sols so that the following may be recommended as a standard stock solution to be used in adjusting the sensitiveness of gold sols.

PREPARATION OF THE STANDARD STOCK SOLUTION

A 1:400 solution of edestin in 10 per cent sodium chloride is prepared by dissolving 0.25 gram of edestin in 100 cc. of 10 per cent sodium chloride solution. After standing about two hours with occasional stirring, the solution is filtered and the volume adjusted to 100 cc. This stock solution is preserved in the ice box (6 to 8 °C) and gives consistent readings over a period of from five to six months. In order to leave a margin of safety, the stock solution should be prepared every three months. For use, this solution is diluted 1:25 with distilled water, yielding a 1:10,000 solution of edestin in 0.4 per cent sodium chloride. It is essential to prepare the latter freshly before each titration.

METHOD OF TESTING THE SENSITIVENESS OF THE GOLD SOL

To each of a series of six test tubes containing the following amounts of 1:10,000 edestin solution—0.01 cc.—0.2 cc.—0.3 cc.—0.4 cc.—0.5 cc.—is added 5 cc. of the colloidal gold sol to be tested. After standing overnight (about eighteen hr.) readings are made as in the test with spinal fluid. If the gold sol is satisfactory, the tube containing 0.3 cc. of diluted edestin solution will yield a '4' reading and the control tube (without edestin) a "0" reading. The usual readings for the series would be

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†Kreidler, William A. and Small, James C. A Method of the Standardization of Colloidal Gold Sols in the Lange Test 17-259, 1931.

‡Edestin H. P. used in this work was that of the Pfanzstiel Chemical Co., Waukegan, Illinois.

TUBE NO	1	2	3	4	5	6
Edestin (1 10,000)	0	0 1 cc	0 2 cc	0 3 cc	0 4 cc	0 5 cc
Colloidal gold (cc)	5	5	5	5	5	5
Typical Reading	0	1	2	4	5	5

If tube No 4 gives less than a "4" reading and precipitation in tubes No 5 and No 6 is not complete, the gold is not sufficiently sensitive and acid must be added. If tube No 4 gives a "5" reading, the gold sol is too sensitive and alkali must be added. In order to determine the proper amount of acid (or alkali) required for adjustment a titration is set up as follows:

METHOD OF STANDARDIZING GOLD SOLS BY TITRATION WITH ACID IN THE PRESENCE OF STANDARD EDESTIN SOLUTION

TUBE NO	1	2	3	4	5
N/1000 HCl (cc)	0 1	0 2	0 3	0 4	0 5
Colloidal Gold (cc)	5	5	5	5	5
Edestin (1 10,000) (cc)	0 3	0 3	0 3	0 3	0 3
Typical Reading	3	4	4	5	5

After standing eighteen hours, the reactions are observed and the tube containing the smallest amount of acid yielding a "4" reading indicates the correction to be made. For example, if the readings on the above tubes are -3 -4 -4 -5 -5 then the adjustment necessary for 5 cc of gold sol is 0 2 cc of N/1000 HCl, or 0 2 cc of N/5 HCl must be added to 1000 cc of gold sol to adjust the reaction correctly.

If the gold sol is too sensitive, a similar titration, but using N/1000 NaOH instead of N/1000 HCl is carried out. From the amount of N/1000 acid or alkali found necessary by these titrations to correct the 5 cc of gold sol used, a simple calculation determines the amount of N/5 acid or alkali required for the adjustment of the bulk of the solution. The volume of N/1000 acid or alkali necessary to correct 5 cc of gold sol is the same as the volume of N/5 acid or alkali necessary to adjust the reaction of 1000 cc of gold sol correctly.

Note. In rare cases, it may be necessary to extend the titration series to more than five tubes, increasing the amounts of N/1000 acid or alkali by 0 1 cc in successive tubes.

It is the practice in this laboratory to set up the three titrations at the same time. If the gold is incorrect, the amount of reagent necessary for adjustment may be determined without further delay. For the past year we have been using this method of preparing the gold sols used in more than 3600 routine tests of spinal fluids. Each lot of colloidal gold was also checked with the spinal fluid method to which reference has been made¹. Results have checked consistently, hence, we present the edestin method for adjusting gold sols as possessing all the accuracy of the spinal fluid method and emphasize the additional advantage of its being more practical in smaller laboratories where positive spinal fluids are obtained with difficulty.

CONCLUSIONS

A standard solution of edestin which precipitates colloidal gold in a manner similar to that of spinal fluids from paretics is described

A method of employing this standard solution in adjusting the different lots of colloidal gold so that they exhibit a uniform degree of sensitiveness in the colloidal gold reaction with spinal fluids is described

This method has been in use for more than a year during which upward of 3600 tests were made and it has been found to be accurate and practical

THE USE OF HYDROGEN PEROXIDE IN THE MICRO KJELDAHL NITROGEN METHOD*

BY VICTOR C MYERS, CLEVELAND, OHIO

IN 1914 I¹ described a slight modification of the Folin-Farmer² micro Kjeldahl nitrogen method for urine which permitted direct nesslerization. After this method had been in use a short time, it became apparent that the procedure could be materially shortened if some oxidizing agent could be added to the sulphuric acid to hasten the final oxidation. A number of reagents were tried, but hydrogen peroxide seemed to be the best suited for the purpose. At that time (1915) Merck's perhydrol was used in somewhat diluted form, since it was found that a drop of the 30 per cent solution was considerably more than adequate for the purpose. The suggestion of the use of the hydrogen peroxide came from Dr. A. R. Rose, who was a member of the staff of the Post-Graduate biochemical laboratory at the time. The use of hydrogen peroxide in the estimation of the nonprotein nitrogen of the blood was described in this JOURNAL in April, 1920,³ and its use in both the nonprotein nitrogen of the blood and the total nitrogen of the urine in the first edition of *Practical Chemical Analysis of Blood* in 1921.³ In a paper which Dr. Rose⁴ published on the micro Kjeldahl method in 1925 he stated that hydrogen peroxide was used in Myers' laboratory before it was mentioned in the literature, but did not credit himself with this suggestion. Credit for the first use of hydrogen peroxide in the micro Kjeldahl method is due to Dr. Rose.

Although perchloric acid is a more active oxidizing agent, it appears to be inferior to hydrogen peroxide when the nitrogen is determined by nesslerization, apparently for the reason that there is greater formation of amines with the perchloric acid. Although amines titrate the same as ammonia they do not give the full color development of ammonia with Nessler's solution.

The reason for calling attention to the matter now is that credit is being given to Koch and McMeekin⁵ for the use of hydrogen peroxide in the micro Kjeldahl method. In September, 1924 they published a paper on a new direct nesslerization micro Kjeldahl method and a modification of the Nessler-Folin reagent for ammonia, being apparently unaware of previous publications on the subject. It is true that they advocated direct nesslerization of their blood filtrates, whereas we only described this for urine, but the hydrogen peroxide was used for the same purpose as employed by us. It might also be noted that their Nessler-Folin reagent is essentially the same and is prepared in essentially the same way as the Nessler solution (Benedict formula)[†] given in

*From the Department of Biochemistry, School of Medicine, Western Reserve University. Received for publication July 2, 1931.

†This formula was supplied us by Dr. S. R. Benedict some months prior to the publication of Folin and Denis in 1916 in which the composition of Nessler's solution was discussed.

Practical Chemical Analysis of Blood in 1921⁷ except for a somewhat stronger alkalinity necessitated by the larger amount of sulphuric acid they used

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THE QUANTITATIVE DETERMINATION OF CHOLESTEROL IN THE BILE*

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ALTHOUGH cholesterol determinations in the blood have been the subject of much study and have now become a more or less routine examination in many laboratories, its measurement in the bile has escaped very extensive investigation. As a result we encountered difficulties in our early attempts to find a satisfactory procedure. From the various methods described in the literature as well as from the excellent advice of Dr. Michael Somogyi we have selected a simple, economical and, we believe, accurate procedure which has been used in a series of experiments on the cholesterol function of the gall bladder.¹

PREVIOUS WORK

Excellent discussions of previous methods can be found in the papers of McMaster² and Meikelbach.³ In general two procedures have been used. The digitonin (gravimetric, Windaus) method of determining cholesterol while perhaps the most accurate, was at once discarded as too complicated and expensive for our use. Its advantage over the simpler colorimetric (Autenrieth-Funk) method, moreover, has been investigated by many workers³ and, for our purposes was insufficient to recommend it. A small series of parallel determinations moreover, between the digitonin and the present method showed close agreement, as is evident from the results described below.

The special problem relating to bile concerns that of extraction in order to obtain a chloroform solution of the cholesterol present, free from the contaminating color of the bile pigments. Elaborate procedures such as used by Doyon and Dufourt⁴ were found unsuitable for routine use. In the most recent study of cholesterol in bile by McMaster² large amounts of ethyl ether were used, 350 cc. for each determination. In our early work we used this method and found we were apt to get a yellowish tinge in the extract, which we assumed was due to some dissolved bilirubin. If we dried our sample of bile on filter paper⁵ and extracted with ethyl ether in a Soxhlet this yellow color was even more marked. The use of large amounts of ether, moreover, was expensive and gave no better results than the method we finally adopted. Fowweather and Collinson⁶ used chloroform directly after adding sodium hydroxide to "fix" the bile pigments. We found that this still allowed too much of the bile pigment to come through which interfered with the final color reaction. Meikelbach³ extracted an alcoholic bile mixture with ether in a separatory funnel. In our hands separation with ethyl ether was always difficult and often impossible which led to our use of petroleum ether which gave no further trouble.

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METHOD

The effect of various conditions on the color reaction was first investigated in a few preliminary experiments by studying the color produced by a standard chloroform solution of cholesterol carried through the standard procedure as described below. It was compared with an inorganic standard prepared by mixing 10 per cent CuSO_4 and 1 per cent $\text{K}_2\text{Cr}_2\text{O}_7$ solutions so as to give an appropriate green color.

For routine determinations a standard cholesterol solution was prepared by dissolving the Merck and Company preparation in chloroform so that 1 cc. of it contained 15 mg. Whenever possible sufficient bile was taken to yield about as much cholesterol as this, so that the unknown would match as closely as possible with the standard. Ordinarily 5 cc. of bile was taken and 20 cc. of 3 per cent KOH (in 95 per cent ethyl alcohol) added and heated on a water-bath for fifteen to thirty minutes in a 100 cc. Erlenmeyer flask, cooled and extracted twice (or 3 times, the result was the same) with petroleum ether (maximum B.P. 80° C.) in a separatory funnel. This extraction was quite easy. In case separation did not occur promptly a few drops of ethyl alcohol were added which layered the two solutions at once.

The petroleum ether extract was then evaporated and the residue taken up in chloroform by repeated extractions totaling not over 7 cc. and gathered in a 10 cc. volumetric flask. Pure acetic anhydride (2 cc.) and concentrated H_2SO_4 (0.1 cc.) were then added, shaken and made up to the mark with chloroform. A similar procedure was carried out simultaneously in another flask with 1 cc. of the standard (made up also to about 7 cc. with chloroform). Ordinarily only 2 or 3 biles were examined at one time with one standard. The conditions of light, time and temperature were the same for the standard as well as the unknowns and comparisons in a colorimeter were made within five to fifteen minutes or as soon as a good green had developed.

We compared the results of this method with that obtained (1) from the direct Soxhlet extraction of bile dried on filter paper, and (2) from the digitonin method as described by Okey. These latter determinations were kindly performed by D. J. Kooyman, for which grateful acknowledgment is made.

RESULTS

Early in our work we noted the influence of certain factors on the development of the green color factors which have been mentioned by previous workers. To evaluate them accurately would have led us too far afield. A number of observations were made, however, which may be briefly described as follows.

Time—The time factor is shown in the accompanying curves (Fig. 1). It is obvious that the maximum color develops rapidly, lasts for five to fifteen minutes, and then gradually fades off. Hence we made readings within this time. The two curves also show that the reaction is faster and more prolonged in daylight than in artificial light. The quality of the color was also different as will be mentioned below. Ordinarily in bright daylight the hue

becomes grey after thirty or forty minutes, whereas in artificial light or darkness it becomes yellowish instead, and after a few hours a pure yellow or brown

Temperature—We noted that the higher the temperature the more rapid the reaction but the more green the quality of the color, unless the light were intense. If one adds the reagents while the flask is immersed in ice water, the color will scarcely develop at all. If one then places it at room temperature it comes on within fifteen minutes but is almost a pure blue with little yellow. By increasing the temperature the blue changes more and more toward green. If one carries out the color reaction in the dark with heated reagents the green becomes almost yellow, even if the time interval is the same.

Light—Daylight was found to produce a more intense color than artificial light or darkness as is shown in the experiments represented in Table I and the curves in Fig 1. The quality of the color is more blue with daylight and more green with artificial light. Indeed, as mentioned above, by developing the

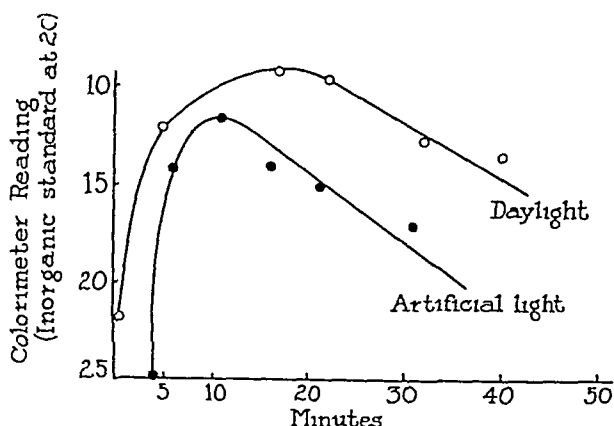


Fig 1—Curves of colorimeter readings of pure cholesterol following standard procedure showing (1) the changes in intensity with time and (2) that daylight causes a more intense and prolonged color than artificial light

color with heated reagents in the dark, one can obtain an almost pure yellow color. Sunlight was found to develop a rapid blue color, which, however, loses its intensity quite rapidly, and becomes a dirty grey.

As a result of these observations we allowed the color to develop at room temperature and usually in ordinary late afternoon daylight. While being measured in the colorimeter artificial light was used but it shone equally on the unknowns as well as the standard. Reagents were added at the same rate to all flasks so as to avoid any great differences in the temperature rise, which occurs when the acetic anhydride and H_2SO_4 is added. The advantages of using a cholesterol standard, therefore, are that whatever factors vary from day to day, they affect the two solutions compared equally.

Duplicate determinations were made with many specimens and found to agree within 10 per cent and often within 5 per cent. It should be noted, however, that better checks were obtained with biles containing much cholesterol. To avoid matching the weak green elicited with samples containing little

cholesterol more bile was taken 10 or even 15 cc. at times. This difficulty in measuring small amounts however is one which applies to all methods.

In general the color obtained with the present method, even for samples containing little cholesterol was a better one and easier to match than the

TABLE I

EFFECT OF VARIOUS KINDS AND DURATION OF LIGHT ON THE INTENSITY OF THE COLOR BY ACTION INORGANIC STANDARD* SET AT 20 REAGENTS AT ROOM TEMPERATURE

EXPERIMENT	KIND OF LIGHT	DURATION (IN MINUTES)	AVERAGE READINGS
1	Moderate daylight	10	10.7
	Darkness	10	13.3
2	Moderate daylight	10	11.0
	Darkness	10	16.0
3	Moderate daylight	15	10.0
	Darkness	15	12.5
4	Artificial light	10	12.0
	Darkness	10	15.5
5	Artificial light	15	10.8
	then daylight	5	9.9
	Darkness	15	12.3
	then daylight	5	10.9
6	Direct sunlight	15	15.2
	Direct sunlight	20	21.5
	Bright daylight	15	9.9

*10 cc. of 10 per cent CuSO_4 (acidified) + 0.1 to 0.5 cc. of 1 per cent KCrO_4 depending on hue desired.

color obtained from bile dried on a filter paper and extracted in a Soxhlet with ethyl ether. A series of such comparisons are represented in Table II, which shows the superiority of petroleum ether over ethyl ether although the preliminary saponification in the former may have been responsible for the better readings. The lower values (larger readings) with petroleum ether probably indicate a greater accuracy since noncholesterol substances have not added to the color.

Table III represents comparative determinations by the present method and by the digitonin method. It will be noted that the values check very well in all but specimen 3 where the gravimetric result is higher. Since color-

TABLE II

COMPARISON OF COLORIMETER READINGS AGAINST A KNOWN CHOLESTEROL STANDARD, OF BILES EXTRACTED WITH ETHYL ETHER (WITHOUT SAPONIFICATION) AND WITH PETROLEUM ETHER (WITH SAPONIFICATION). THE FORMER READINGS IN MOST CASES ARE SMALLER, INDICATING A STRONGER COLOR AND HENCE A LARGER VALUE THAN THE LATTER.

BILE		PETROLEUM ETHER		ETHYL ETHER	
SOURCE	NUMBER	READING	MATCH	READING	MATCH
Human	1	4.2	good	3.7	good
Human	2	15.0	good	11.5	poor
Human	3	13.0	good	12.5	fair
Human	4	4.7	good	4.8	good
Human	5	1.3	good	1.5	good
Human	6	18.7	good	16.5	too yellow
Dog	1	10.20	good	7.5	too yellow
Dog	2	19.5	fair	"	too yellow to read
Dog	3	17.0	fair	"	too yellow to read

imetric results are usually higher, we went back over the steps used in this specimen and found that the filtrate before precipitation with digitonin had inadvertently been left exposed to the air for several weeks and that the high value may therefore have been due to the presence of dust in the final precipitate

TABLE III

COMPARISONS OF CHOLESTEROL DETERMINATIONS OF VARIOUS BILES BY THE PRESENT METHOD AND THE DIGITONIN METHOD

SOURCE	PRESNT METHOD (MG /C C)	DIGITONIN METHOD (MG /C C)
1 Human gall bladder bile	2.21	(1) 1.92 (2) 2.03
2 Human hepatic bile	(1) 0.96 (2) 1.01	1.05
3 Dog gall bladder bile	(1) 0.69 (2) 0.68	0.91 (see text)
4 Dog hepatic bile	0.083	0.087

The actual values obtained with this method are being reported for both hepatic and gall bladder biles from human beings as well as dogs.⁸ Hepatic bile, collected aseptically from dogs varied between 0.04 mg/c c to 0.20 mg/c c with an average at about 0.1 mg/c c, values agreeing with those recently reported by Enderlen, Thannhauser and Jenke,⁹ who used the digitonin method, and also those by Stern.¹⁰ They were somewhat lower than those found by McMaster.² Gall bladder bile from dogs contained much more cholesterol, even when as dilute as liver bile. The values varied between 0.6 mg/c c to 2.4 mg/c c in a large series of isolated determinations, averaging about 1.2 mg/c c. In the human being, the values were still higher but here too the specimens removed from the gall bladder were much higher than those obtained from the common duct.⁸ The specimens from the gall bladder agree rather well with the values reported by Fowweather and Collinson.⁶

COMMENT

The method described herein for the quantitative determination of cholesterol in bile has the advantage of extreme simplicity, requires but little reagents and from the data presented, seems to be as accurate as the results obtained with other methods.

The importance of saponification has recently been emphasized by Oker and others since noncholesterol substances are capable of producing the characteristic green color reaction but that they are not extractable after saponification. The use of an alcoholic solution enables the extraction with petroleum ether to be easily and rapidly carried out.

The yellowish discoloration mentioned by most workers was encountered only in specimens very weak in cholesterol and could thus be partly avoided by using more bile for extraction. It was otherwise apt to occur only when the temperature was high and when the light was poor, as discussed in some detail above. With suitable precautions, therefore, it could usually be avoided.

SUMMARY

A simple and economical method for the quantitative determination of cholesterol in dog and human bile is presented which seems as accurate as and in many ways more satisfactory than previous methods. Observations are also reported on the influence of temperature, light, and time on the development of the color reaction used in the method.

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NONELASTIC BULB FOR PIPETTES*

By JOHN W. WILLIAMS, M.D., NEW ORLEANS, LA

DIFFICULTY in manipulation of fluids by means of the rubber teat fitted to the pipette has led us to devise a nonelastic bulb to be used in place of the rubber teat. This bulb obviates the necessity of continued pressure when different amounts of fluid are being measured. It is easily cleaned and

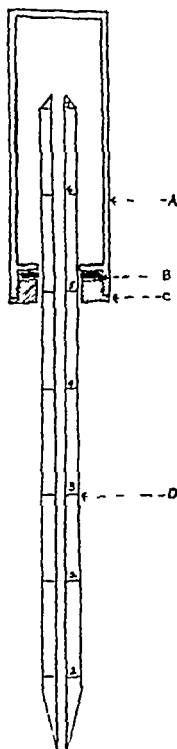


Fig. 1

sterilized. It slides with ease on the pipette the calibrated distance desired, expelling or drawing up a measured amount of fluid. It will not easily deteriorate or corrode and is comparatively cheap to manufacture. The bulb may be made of glass or other suitable material of the desired shape, size, and capacity to fit a standard pipette.

DESCRIPTION OF CROSS SECTION

(A), chamber, responsible for pressure changes at lower end of which are two flanges, one at right angles to and fitting on to the pipette, the other

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threaded and parallel to it. A piece (*C*), shaped as illustrated and fitting the pipette snugly screws into (*1*). By means of these threads, (*B*) which act as a replaceable washer, may be compressed or loosened, thus allowing variation in tightness of fit to pipette (*D*).

This device may be varied so that (*C*) is made continuous, with the flange of (*1*) and inlay of suitable material (*B*) made in the space between (*1*) and (*C*) or the right angle flange of (*1*) made of sufficient width and tightness to fit the pipette in an air tight manner and the necessity of (*1*) and (*B*) eliminated.

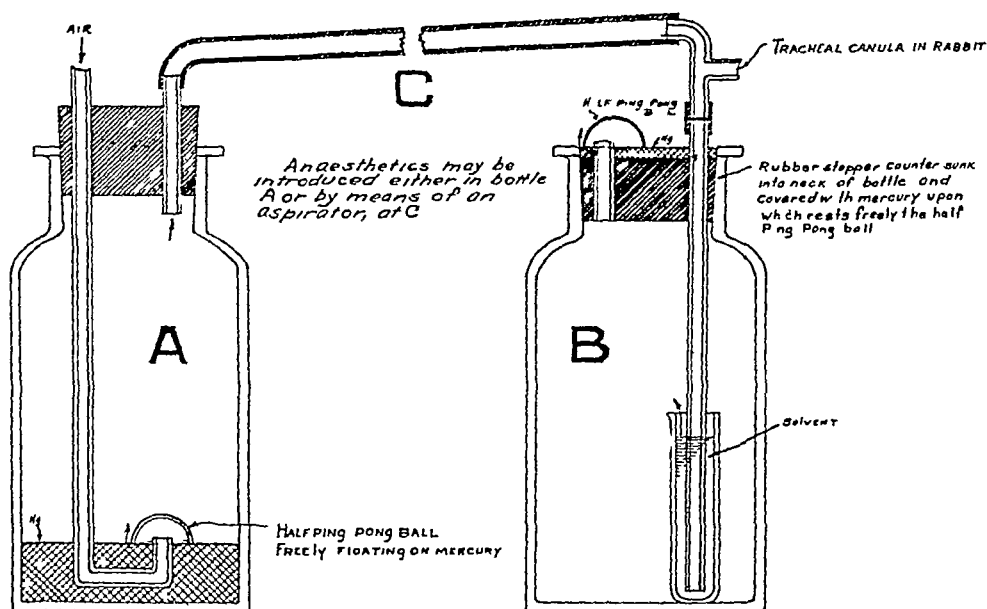
The advantage of the bulb is that it can be fitted to one pipette after another much more quickly than can the rubber test. This will greatly speed up work in which a series of pipettes are to be used and will obviate loss of patience incident to the difficulty often experienced in the adjustment of a rubber test to a pipette. To make the adjustment easier it is desirable that the upper end of the pipette be rounded as illustrated.

The device can be made so that a ring for the first finger may be attached to the top of (*1*). In this case the thumb and second finger will grasp the pipette and (*1*) will be moved up and down on the pipette by means of the first finger.

A SIMPLE APPARATUS FOR ABSORBING SUBSTANCES FROM THE EXPIRED AIR OF LABORATORY ANIMALS*†

By WITHROW MORSE, BRISTOL, PA

THIS apparatus can be made from common laboratory supplies, supplemented by a ping-pong ball obtainable at sporting goods stores. The ball is divided into its two halves at its equator and each half rested upon a mercury surface as indicated in the drawing. In order to move the column of air past these valves, it is necessary to have a force only sufficient to raise their



APPARATUS USED TO INSURE COMPLETE MIXING OF EXPIRED AIR FROM EXPERIMENTAL ANIMAL WITH SOLVENT

Fig 1

relatively insignificant weight. In inspiration, the half-ball fits down into the mercury and prohibits all movement of air past it.

Anesthetics may be introduced directly into the containers, or, if desired, into a "U-tube" inserted into the connecting tube.

The apparatus is equally efficient in absorbing substances from the expired air of a white rat and from a large mammal such as the dog or man himself. In the case of small mammals, the column of absorbing fluid in the

*From the laboratories of Rohm and Haas Co. Inc.

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†Demonstration before the Amer. Soc. Phil. Chemists, McGill University, April 1931

first container should not impede the passage of air—this is accomplished by keeping the column as short as possible.

For a large mammal it may be essential to use an absorption tube (closed, expanded at the end into a bulb and holes provided in the bulb) owing to the great pressure of expiration.

The absorbing liquid of course depends upon the character of the substance sought. Readily volatile substances and absorbing agents should be protected by a layer of some reagent such as mineral oil, in order to reduce surface tension when applied to the receiving vessel.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

LEUKOCYTES The Staff Count in Infectious Disease, Weiss, A Arch Int Med 48 399, 1931

A careful review of the data prepared from about 20,000 examinations of the blood performed during the past five years yields the following conclusions

1 The leucocytosis caused by acute infections is primarily the result of stimulation of the bone marrow. This reaction of the bone marrow is a nonspecific, biologic phenomenon that depends not only on the type of organism, but on the degree of irritation caused by bacterial toxins. The neutrophilia thus brought about shows a varying percentage of immature or staff neutrophils, depending on the severity of the toxemia and the ability of the bone marrow to respond to it. A careful morphologic examination of serial blood films taken during the course of an infection will demonstrate that

a The peak of the staff count and the height of the infection usually coincide

b The peak of the staff count drops as soon as the infectious process is removed or overcome

c The persistence of a high staff count usually means a complication

d The persistence of a high staff count may mean that the infection is becoming subacute or chronic

e The persistence of a high staff count without the possibility of removing the infectious focus usually indicates a fatal outcome

f The presence of a high staff count early in the course of lobar pneumonia usually is indicative of a fatal outcome

g The curve of the daily staff count is more accurate as an indication of the course of the infection than the chart of the temperature

h The staff count is more reliable than the leucocytic or polymorphonuclear count

2 Concomitant with the sharp drop in the staff count are a sudden increase in the number of monocytes and a return of the eosinophils into the circulation

3 In subacute or chronic infections, one finds a persistence of an elevated staff count with an increased number of monocytes and lymphocytes

4 Lymphopenia, which is present during the neutrophilic phase of acute infections, is replaced by lymphocytosis during the period of convalescence and healing. In subacute or chronic infections, the lymphocytes are usually increased

For those who employ this method of morphologic examination of the blood, it is extremely important to bear in mind that a single report of a high staff count does not spell a fatal prognosis. Daily morphologic examinations of the cell are of inestimable importance, for it is on the curve that the prognosis of the case depends. It must be remembered that the changes in the blood are always to be considered conjointly with the complete clinical findings. It is also advisable to bear in mind that outstanding changes in the blood cannot be disregarded because of the lack of clinical corroboration. Nevertheless, it is also true that a definite clinical picture cannot be negated by a lack of confirmatory hematologic observations. Last, but not least, it must always be borne in mind that examination of the blood cannot always be employed as a means of diagnosis. Although one usually finds a definite biologic chain of leucocytic interreaction in infections, every now and then, owing to some unrecognizable cause, the blood picture fails to indicate the patient's condition. One must not forget that even now the reason for the appearance of the various cells in the circulation is not known, and therefore no cause can be ascribed for their failure to appear. These hematologic failures must not serve to discourage the clinician or the laboratory

worker, but should spur him on to deeper and more exact observations. Careful serial morphologic examinations in which use is made of the neutrophilic subclasses are of inestimable value in the hematologic study of all infectious disease.

DIABETES Study of the Five Hour Dextrose Tolerance Curve in Treated Diabetic Patients Ralli, E. P. and Shannon J. *Am J Med Sci* 182: 95, 1931

SUMMARY

1 The blood sugar curve following the ingestion of 100 gm. of dextrose was studied for a five hour period instead of the customary three hours in normal individuals and in mild, moderate and severe diabetics.

2 In the normal group the blood sugar reaches its height in from 0.5 to one hour. The blood sugar returned to a normal level at the second or third hour.

3 In the group of mild diabetics the return of the blood sugar to its starving level was at the third or fourth hour. The greatest hyperglycemia was at the first or second hour.

4 In the moderately severe group the return of the blood sugar to its starving level was delayed to the fourth or fifth hour. The greatest hyperglycemia occurred at variable periods.

5 In the severe group the blood sugar was markedly elevated for the entire five hour period.

6 There is evidence presented that a starving blood sugar level of over 160 mg. suggests that the patient will not return to this level within a period of five hours following the ingestion of 100 gm. of dextrose.

CONCLUSIONS

1 It is suggested that the blood sugar level taken four hours after a meal is a better index of the severity of the diabetes than the starving blood sugar.

2 The severity of the diabetes as classified by clinical criteria was supported in all but 3 cases by the glucose tolerance test.

3 The five hour test is found to give more valuable information in many cases than the three hour test.

BACTERIOPHAGE Factors in the Preparation of Keller, M. J. *Bacteriol* 22: 199, 1931

The method following was found suitable for the preparation of bacteriophage in large amounts.

The method has two prerequisites. A bacteriophage having a lytic titer of 10^{-6} or higher, and an inoculum prepared as follows: (a) A loop of the homologous organisms is inoculated into a tube of broth (about 6 c.c.), P_{H} 7.6, and incubated for six hours at $37^{\circ} C$. (b) One part of this culture is added to a thousand parts of broth and incubated one hour at $37^{\circ} C$. This one hour culture is referred to as the inoculum in the following outline.

1 To 100 c.c. of bacteriophage is added an equal amount of inoculum and the mixture is incubated for twenty four hours at room temperature.

2 If this mixture also is clear, an equal amount of (400 c.c.) of inoculum is added and incubated again for twenty four hours at room temperature. This "doubling" procedure is continued until the desired volume of bacteriophage is obtained.

3 At this point, approximately 100 c.c. of a six hour broth culture per liter of mixture is added and further incubated for two days at room temperature. The mixture should now appear opalescent. If it is turbid, it should be filtered through a Berkefeld candle and the clear filtrate reinoculated with less than 100 c.c. of a six hour culture, if it is clear, an amount larger than 100 c.c. of culture is employed for the inoculation. The aim is to add

an amount of culture that will produce an opalescent mixture within two days of incubation at room temperature. This step is based on an observation that bacteriophage filtrates remained clear if, before final filtration, they had given rise to an opalescent or a flocculent form of resistant growth which was permitted to remain in the bacteriophage for some days at room temperature.

4 Opalescence having been attained the mixture is incubated at room temperature for five to seven days, after which period it is filtered.

5 The clear filtrate is checked for lytic potency and for possible contaminations, and if satisfactory is ready for therapeutic use.

Special Conditions If after step 1 the mixture shows opalescence, it is incubated without filtration for about seven days to increase the titer, after which period it is filtered and the process continued with step 2. If after step 1 the mixture is turbid, indicating relatively low lytic potency, it is filtered after four days' incubation at room temperature, and an inoculum equal to half the volume of mixture, instead of the total volume, is added and incubated for twenty-four hours; if this mixture remains clear, the process is continued with step 2; if opalescent or turbid, the steps are repeated as given in this paragraph.

PERTUSSIS Isolation and Cultivation of *H. pertussis*, Bailey, J. H. Am. J. Pub. Health 21: 1144, 1931

The following modification of the Bordet Gengou medium is recommended.

Paired potatoes are cut into thin slices. To one kg. of potato are added 2,000 cc. of water and 80 cc. of cp. neutral glycerin. This is boiled over a free flame until the potato falls to pieces, the water lost by evaporation being replaced. The material is then rubbed through a sieve and strained through a towel. The expressed juice is then adjusted to a P_H of 7. To 1 part of neutralized juice (it is rather thick) is added 3 parts of 0.6 per cent sodium chloride solution. The material is then distributed in 200 cc. lots into 500 cc. flasks. To each flask is added 8 to 10 gm. of agar. The flasks are then plugged and set in the ice chest overnight. If powdered agar is used it is not necessary to store overnight. The flasks are then autoclaved at 15 pound pressure for thirty minutes. When plates are to be poured the agar is melted and then cooled to about 40° C. Two hundred cc. of sterile defibrinated horse blood, warmed to about 40°, is added and thoroughly mixed. To the resulting mixture 6 cc. of sterile N/2 lactic acid is added and mixed. Plates are then poured and one plate is incubated for two to three days to test the sterility. This plate is not used again.

Plates of this medium may be stored in the ice chest for two weeks. It is important that the agar be not too hot at the time the blood is added, as *H. pertussis* grows only on unaltered hemoglobin. Indeed this fact has been used as a method for identifying *H. pertussis* and differentiating it from *B. influenza*, the latter grows on chocolate agar, the former will not until after several generations on artificial medium.

MEDIUM for Isolation and Cultivation of Bacteria in the Filterable State, Kendall, A. D. Northwestern Univ. Bull. 23: 8, 1931

The "K" mediums described below were successful in the isolation and cultivation of organisms in the filterable state from various sources from which no growth could be obtained with the usual methods.

Fresh tissue from the animal or human body is thoroughly extracted with 95 per cent alcohol. Intestine has been used chiefly for this purpose. As a routine, the intestine is opened, cleaned, and ground in a meat chopper and immersed at once in about four volumes of alcohol. Extraction at 37° C. is practiced for one or preferably two days with occasional stirring. The alcohol is then removed, and fresh alcohol added. This is repeated twice, making three extractions in all. This procedure removes water, alcohol soluble extractives

and some fat. The dry tissue residue is next reextracted with benzol to remove most of the remaining lipoidal constituents. The benzol is removed first by filtration, then by an air current. The extracted material is finally reground to a fine powder. It will keep indefinitely in a dry stoppered container. This dried tissue residuum is the nutritive basis for the medium about to be described. It is not necessary to limit the tissue to intestine; brain, liver, kidney, spleen and heart have been used, although in general, liver, kidney or spleen have proved to be rather less suitable than intestine. Heart muscle has been fairly satisfactory. It may be ground very fine in a meat chopper and dried rapidly in a warm air current in lieu of alcohol extraction, though alcohol extracted tissue appears on the whole to be rather better than air dried tissue. Tissue from any animal may be used. Hog intestine however has been distinctly more suitable than rabbit intestine, and rabbit intestine has appeared to be more favorable than dog intestine. Human intestine, which was not available when the early studies were made, is under investigation at present.

A small amount of dried intestine, two per cent by weight, or thereabouts, is placed either in a test tube or a flask of the proper capacity. Normal saline solution, or somewhat better, tyrode solution is added (tyrode solution, as used in this work has the following composition: NaCl 8.0 gr, KCl 0.2 gr, CaCl₂ 0.2 gr, MgCl₂ 0.01 gr, NaH₂PO₄ 0.05 gr, NaHCO₃ 0.2 gr, Glucose 0.5 gr, distilled water 1000 cc.) Physiologically normal KCl may be used in place of physiologic saline, or distilled water may be substituted for tyrode solution. Generally speaking, however, neither KCl nor water is as satisfactory as normal saline or tyrode solution. It is best to introduce the dry tissue first, then the solution. The tissue is thereby wetted, and does not float and form during steam sterilization. It will be noted that commercial peptone and meat extractives are rigidly excluded from K medium, this is very important. The medium thus prepared is usually slightly acid after autoclaving, hence it is necessary to add a small amount of NaHCO₃, usually one-half gram per liter is sufficient. The final reaction should be from P_H 7 to P_H 7.4. If the alkalinity exceeds this amount the medium becomes distinctly more cloudy, and there is evidence of disproportionate decomposition of the nitrogenous constituents during sterilization in the autoclave. This is to be avoided for reasons that will appear below. The medium thus prepared is slightly turbid and has a tissue sediment. It is more turbid when normal saline is used as the diluent, less turbid when tyrode solution is employed.

A "clear" K medium may be prepared in the following manner: two per cent by weight of dried intestine or other tissue is thoroughly minced, preferably with the proper amount of tyrode solution, and heated at 50° C with frequent stirring for one hour. The solution is allowed to settle, and the supernatant, somewhat cloudy part is filtered through a sterile filter paper, which removes the greater part of the suspended substances. The filtrate in turn is passed through a sterile Berkefeld W filter, using the set up described elsewhere (Kendall Bacteriology, 3rd edition, figure 20, page 222). With this apparatus, there is almost no hazard from contamination during the entire process of filtration and the filtrate may be distributed in test tubes or in flasks as may be convenient. The chief advantage of the clear K medium rests in its applicability to the dark field examination of bacteria that may be cultivated in it. Under the dark field, the uninoculated medium is found to contain many very small, faintly greenish yellow granules, and varying numbers of larger, bright yellow granules. The regular K mediums contain multitudes of granules of varying sizes and degrees of brightness. Bacteria in the filterable state also appear as brilliant yellow granules, but comparison with uninoculated K medium will usually afford points of differentiation.

The "clear" K medium is not recommended for isolations of bacteria or for pure culture perpetuation, there is always a possibility that bacteria in the filterable state may escape the action of heat at 50° for one hour, and remain viable in the filtrate. Many, if not most, bacteria in the filterable state will pass through a Berkefeld W filter in numbers sufficient to cause confusion later on. A reasonably satisfactory substitute for the "clear" K medium, which possesses the advantage of unequivocal sterility, may be had by filtering steam sterilized K medium, prepared as above indicated, through sterile filter paper to remove the grosser particles, then through a Berkefeld W filter.

In spite of the prolonged extraction with alcohol, there appear to be some saccharoid substances left in the tissues which interfere very materially with the study of fermentation reactions in the K medium. This is a disadvantage that has so far proved unsurmountable. It is frequently desirable, however, to add from 3 to 5 per cent of glycerin to K medium before steam sterilization. These and other factors in the chemistry of the K medium will be discussed later. Suffice it to say here that it contains protein in colloidal solution. Some of this protein precipitates when vigorously active organisms, as the gas bacillus, are grown in it for the first transfer. It is significant that second transfers, even of the gas bacillus, in K medium usually do not precipitate the protein constituents. The organisms are changing at this time to the filterable state, a condition in which chemical activity is apparently materially modified. This K medium is in acceptable probulum for the bacteria studied so far, which include many types ranging from those isolable from the blood of persons having influenza, common cold, rheumatic fever, and arthritis, to those microbes cultivable in ordinary laboratory mediums.

Just how much degradation the protein constituents of the K medium undergo during heat sterilization cannot be decided at this time. If, however, the mediums are carefully steam sterilized, the organisms thus far studied grow very well.

Thus far, experience has clearly shown that a single exposure to 15 pounds' live steam pressure for twenty minutes in the autoclave is ample to afford sterility, although as a matter of precaution two exposures each of fifteen minutes to live steam at 15 pounds' pressure with a twenty four hour incubation period intervening has been practiced in those mediums put up in flasks to receive 10 cc of blood for blood cultures. These flasks usually contain about 2 gm of tissue and approximately 100 cc of tyrode solution. Test tubes containing but a small amount of extracted tissue, suspended in 10 cc of tyrode solution are sterilized but once as a matter of routine. Controls should always be carried along from the same lot of medium to afford additional information as to the sterility of the uninoculated tubes.

THE USE OF K MEDIUMS

The degree of turbidity of various lots of K medium varies somewhat, hence it is always helpful to incubate an uninoculated tube (or flask) for purposes of comparison with the inoculated one. This is usually less essential if ordinary bacteria in the nonfilterable state are being investigated, but for blood or spinal fluid cultures it is very helpful. Further more, in making blood or spinal fluid cultures, it is best to have the ratio of blood or spinal fluid to K medium rarely exceed 1:10, that is, one volume of blood to 10 of K medium. Anaerobic incubation at 30° C as above indicated, usually gives the most consistent results. Cloudiness, which is the most common sign of growth, does not ordinarily become apparent before the seventh to the tenth day, unless bacteria are present in the nonfilterable state, and therefore cultivable directly upon enriched ordinary mediums. Here evidence of growth may be had often within the second to the third day, or sooner. When cloudiness is unequivocally present, transfer to fresh K medium may be made, with excellent prospects for successful subculture. It is always well to make a subculture from the original culture in ordinary, peptone containing medium, as well as in the K medium. This is also advisable in the original culture from the patient's blood or spinal fluid. These cultures should be kept for several days, as experience has shown that two weeks or more may elapse before growths appear, though in the group of diseases discussed above, both sets of cultures in artificial mediums, even when enriched with blood, will usually be negative. Growth takes place readily, even though slowly, in K medium, however.

REVIEWS

Books for Review should be sent to Dr Warren T. Vaughan Professional Building
Richmond Va.

Nephritis Its Problems and Treatment

(For review see editorial page 292 this issue)

Allergic Diseases, Their Diagnosis and Treatment[†]

ALTHOUGH the first edition of this work is but four years old the third edition represents almost entirely a new volume since it has been built up to nearly twice the original size. The rapidity with which the first two editions were exhausted and the progressive increase in size of the volume are good indications of the increasing interest in the subject and the need for frequent revision and additions due to rapidly accumulating new information.

The author has added in this last edition a discussion of allergies other than inhalant allergies, particularly migraine, urticaria, and eczema. This has necessitated a change in the title from "Hay Fever and Asthma" to "Allergic Diseases." Since the allergic aspects of these various maladies are so interrelated this appears to the reviewer to be a most desirable change.

The author's contributions to allergy, particularly his pollen survey of the midwestern section of the continent, and his contributions on "allergy and intelligence" lend an authority to the book that justifies the rapid appearance of three editions.

A Text-Book of Medicine[‡]

THE review of the first edition of this volume which appeared in 1927 stands equally well for the second edition. Osler's Textbook which stood preeminent for so many years was primarily the experience of one most unusual man supplemented by the experiences of others. Based as it was upon the great personality of the author, the question at once arose as to whether any successor could keep the volume up to date. Dr Cecil has solved the problem in an equally satisfactory way and possibly a more permanent one in that instead of endeavoring to write an entire volume based on personal experiences, he has filled it with a large staff of contributors, each writing on that phase of medicine in which he has been especially

*Nephritis Its Problems and Treatment. By T. Izod Bennett M.D. (London) F.R.C.P. Physician in Charge of Out-Patients, Middlesex Hospital; Physician to the Royal National Orthopaedic Hospital; Late Beit Memorial Fellow for Medical Research, etc. Cloth. Pages 94. Oxford University Press, American Branch, New York, 1929.

†Allergic Diseases, Their Diagnosis and Treatment. By Ray M. Balveat M.A. M.D. F.A.C.P. Lecturer on Allergic Diseases, University of Oklahoma Medical School; Consulting Physician, St. Anthony's Hospital and to the State University Hospital; President-elect of The Association for the Study of Allergy; Director of the Balveat Hay-Fever and Asthma Clinic, Oklahoma City. Illustrated with 57 engravings including 4 in colors. Third Edition, Revised and Enlarged. Cloth. Pages 395. Philadelphia, F. A. Davis Company, 1930.

‡A Text-Book of Medicine. By American Authors. Edited by Russell L. Cecil, A.B. M.D., Sc.D. Assistant Professor of Clinical Medicine in Cornell University; Assistant Visiting Physician to Bellevue Hospital, New York City; and Associate Editor for Diseases of the Nervous System, Foster Kennedy, M.D. F.R.S.E. Professor of Neurology in Cornell University; Head of Neurological Department, Bellevue Hospital, New York City. Second Edition, Revised and Entirely Reset. Cloth. Pages 1792. Philadelphia and London, W. B. Saunders Company, 1930.

NOTE. In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

interested This materially simplifies the problem of revision, since the individual contributors are intimately conversant at all times with advances in their own fields

The second edition measures up to all expectations

Food Allergy*

WITH the development of the allergic concept as the cause for certain diseases the method of skin testing rapidly came into prominence The two diseases recognized as being preeminently allergic were asthma and hay fever As the studies progressed, it became increasingly evident that the outstanding cause of these two diseases was the inhalant allergen Some cases of vasomotor rhinitis associated with food sensitization were noted but these were decidedly in the minority and attention was concentrated chiefly on the inhalant allergens Furthermore, it was observed with increasing frequency that even though a person might be definitely sensitive to some offending food the skin test was not infrequently negative As a consequence the major interest has been given to the inhalants although to be sure ingested allergens have not been altogether neglected

Rowe, whose interest in the food allergens has never lagged, set himself to the task of determining if possible why skin reactions to allergenic foods are negative, and how the study of an allergic case may be successfully carried through in spite of negative reactions to foods

This volume represents the cumulative experience of Doctor Rowe together with a very comprehensive review of the literature on food allergy Since foods are also responsible at one time or another for all of the other clinical allergic manifestations, and since in connection with the inhalant allergies the writer has incorporated discussions of inhalant allergy, diagnosis and treatment, the volume represents a comprehensive discussion of clinical allergy in general, with emphasis on food sensitization

For the clinician who is looking for material of practical reference value the following are to be mentioned specifically the author's description of his own elimination diets and his discussion of the methods of dietary treatment recommended by others interested in this field an abundance of carefully worked out recipes for individuals who must be on wheat less diets, on milk free diets, egg free diets and even for those who must avoid wheat, eggs and milk

Following discussion of diagnostic methods in the study of the food allergies, the author takes up the symptomatology of food allergy including gastrointestinal allergy and the other allergies, bronchial asthma, eczema, dermatitis, angioneurotic edema, migraine, allergic toxemia, perennial hay fever, and certain other less frequent conditions

The chapter devoted to Food Allergy in Infancy and Childhood is most important since the prevention of much suffering depends upon the early recognition of allergy The final section is devoted to a very comprehensive summary of the literature on food allergy

Asthma and Hay Fever in Theory and Practice†

THIS is a volume whose advent allergists have been awaiting for some time It has been written by three men preeminent in their field, each contributing a separate and distinct section The first section, by Dr Coca, deals with the experimental and research phases of

*Food Allergy Its Manifestations Diagnosis and Treatment With a General Discussion of Bronchial Asthma By Albert H. Rowe M.S. M.D. Lecturer in Medicine in the University of California Medical School San Francisco Calif. Chief of the Clinic for Allergic Diseases of the Alameda County Health Center Oakland Calif. Consultant in Allergic and Metabolic Diseases Highland Hospital President of the Association for the Study of Allergy 1927-1928 Cloth Pages 442 Lea and Febiger Philadelphia 1931

†Asthma and Hay Fever in Theory and Practice Part I Hypersensitiveness Anaphylaxis Allergy By Arthur F. Coca, M.D. Professor of Immunology Cornell University Medical College Clinical Professor in Medicine-elect New York Post Graduate Medical School Editor of The Journal of Immunology Part II Asthma By Matthew Walzer M.D. Instructor in Applied Immunology Cornell University Medical College Deputy Attending Physician Clinic of Applied Immunology New York Hospital Chief of Allergy Clinic Jewish Hospital of Brooklyn Part III Hay Fever By August A. Thommen M.D. Lecturer in Medicine University of Bellevue Hospital Medical College Director of the Allergy Clinic Medical College Dispensary New York University Cloth Pages 551 Charles C. Thomas Publisher Illinois and Maryland 1931

allergy, anaphylaxis and immunity, the second section by Dr Walzer is on asthma, and the third by Dr Thommen on Hay Fever. Coe's presentation of the laboratory aspects of the problem brings our understanding of the phenomena of anaphylaxis quite up to date. Into it he incorporates the many constructive observations that have come from his own laboratory but never to the exclusion of other contributions nor does he force them into a position of more prominence than they deserve. From his position as a pioneer in immunology he has watched the subject grow and has contributed greatly to its advances.

While his section deals primarily with the experimental researches, there is a correlation throughout the discussion with the recorded clinical observations.

For the man who is looking for something primarily practical his chapter on The Preparation of Extracts and Solutions for Use in Testing and Treatment, is altogether complete and up to date. While Dr Coe has made several contributions on this phase of the problem in the past, in *The Journal of Immunology*, this chapter presents the most recent of his methods and should be invaluable to the allergist who is properly equipped to prepare his own extracts.

The section by Dr Walzer on Bronchial Asthma is a complete monograph in itself. Following a very comprehensive historical survey, the author presents a critical analysis of all cases autopsied as deaths from uncomplicated asthma, reported to date. The clinical survey includes discussion of treatment. The last section of his monograph presents the most comprehensive encyclopaedia at present available on allergens, and the substances into which they may be incorporated unknown to the patient.

The section on Hay Fever, likewise a monograph, by Dr Thommen, is similarly most complete in itself. The historical survey is excellent and goes into far more detail than any of the other available reference volumes. The section on the botany of plants which cause hay fever fills a need which has been badly felt for some time. It is profusely illustrated with gross specimens and photomicrographs of pollens. The final section deals with treatment.

Recent Advances in the Study of Rheumatism*

THE senior author in this review of the Recent Advances Series which is being published by Blakiston is well known for his pioneer work on rheumatism. Dr Poynton was one of the earliest investigators on the bacterial etiology of arthritis. In this volume the authors contribute a critical review of the recent investigations that have been made toward the study of the etiology of acute rheumatic fever and chronic arthritis. In their presentation they incline toward the acceptance of the evidence that bacterial infection plays a major part although they accept the equally definite evidence that metabolic derangements accompany the rheumatic state. The prevailing tendency today is to consider rheumatic fever as due to streptococcus infection and probably due to a variety of streptococci rather than to one specific organism. The weight of evidence indicates a state of allergy to the infecting streptococcus, and, once the allergic state has been developed, a group sensitization to a number of streptococci. While the most convincing evidence to this end has been produced in the study of rheumatic fever, the analogy in chronic arthritis is brought out and the possibility of allergy playing a part along with streptococcus infection in chronic arthritis is further discussed. In this latter disease, however, organisms are found much more frequently in the joints and in the blood than in acute rheumatism. The authors do not accept Crowe's staphylococcus as a cause of arthritis.

The review is altogether inclusive and the authors are to be congratulated on their ability to put as much information and as broad a discussion of this great field into as small a space as they have succeeded in doing.

*Recent Advances in the Study of Rheumatism. By Frederic John Poynton, M.D. F.R.C.P. (Lond.) and Bernard Schlesinger, M.A. M.D. (Camb.) M.R.C.P. (Lond.) 25 Illustrations. Cloth. Pages 313. Philadelphia: P. Blakiston's Son & Co., Inc. 1931.

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EDITORIAL

The Treatment of Nephritis

THE problem of therapy in nephritis depends for its answer upon the answer to the question, What are we treating? There is still much that we do not know regarding the nature of nephritis and in many respects the treatment still remains empirical. From time to time new remedies have been suggested usually based upon a new theory and urged for use chiefly in an attempt to prove the validity of the theory. Thus today we observe men urging the alkaline diuretics and others equally insistent on the value of acid salts. Curiously both procedures produce results in individual cases.

In briefly reviewing the recent concepts of therapy we will for convenience consider the subject under the following headings: Glomerular nephritis, uremia, edema, and arteriosclerotic nephritis accompanying essential hypertension.

GLOMERULAR NEPHRITIS

Of all these conditions glomerular nephritis is the only one in which we consistently observe true inflammatory reactions in the renal parenchyma. Almost invariably it is associated with bacterial infection and as a rule it is de-

pendent upon either some primary focus of infection elsewhere in the body or an antecedent acute more or less generalized infection. Glomerular nephritis may be focal in distribution or it may be diffuse. It may be acute or chronic. The outstanding urinary finding which distinguishes it from the other types of nephritis is the presence of blood and usually pus. The primary requisite in treatment is naturally to remove the original source of infection in so far as is possible. This involves a thorough study for infectious foci.

The prophylactic treatment is of decided importance since in every acute infectious disease we must bear in mind the possibility of glomerular nephritis as a sequel. The work of Osman and others who claim that the administration of alkalis during fever leads to an important diminution in the number of cases of subsequent nephritis is as yet unsubstantiated but is of the greatest importance if correct. Such treatment amounts to the administration of bicarbonate and citrate during the first weeks of any severe infectious fever in sufficient dosage to render the urine freely alkaline. The same may be accomplished by selecting foods which tend to produce an alkaline urine such as milk, the citrus fruits, apples, bananas, potatoes, melons, peaches, pears and beans, cabbage, carrots, cauliflower, celery, etc. and the avoidance of acid producing foods such as wheat flour, oatmeal, rice, eggs, meat, fish, fowl and oysters. This treatment carries with it apparently no risk and appears to be of distinct prophylactic value.

Where the kidney is already involved in a glomerular nephritis, especially the diffuse type as much rest for the organ as is possible should be obtained by limiting the food intake to the minimum necessary for the maintenance of nitrogenous equilibrium. For a maintenance diet an adult should receive not more than 50 or 60 total gm of protein daily. Tables are now available in which the protein value of average helpings of different foods is recorded, which greatly facilitates the accurate control of the diet. In an acute glomerular nephritis as in uremia the protein intake should be still further restricted to about 25 or 30 gm daily.

Where glomerular nephritis has developed, a slightly alkaline urine probably represents the minimum degree of renal activity and foods and alkali should be adjusted to this end.

In focal glomerular nephritis the renal manifestations are usually so mild that they do not require special treatment. The treatment consists primarily in removing the original source of infection.

UREMIA

Uremia represents a profound disturbance of the chemistry of the body fluids as the result of the failure of the kidneys to perform their normal function. At the present time there is no conclusive evidence that uremia is associated with the presence of a foreign toxin or poison. It merely represents the accumulation of normal waste products. The proportion of accumulation of these products depends upon the normal concentrating capacity of the kidneys. Thus the kidneys normally can concentrate urea about 65 times, calcium only 15 times. Loss of concentrating capacity will show up in measurements of urea much sooner than in measurements of calcium. Between these two extremes

we find that phosphate is concentrated 50 times, creatinine 35 times and chloride 15 times

The treatment of uremia by promoting excretion through other channels is logical. The bowels should be kept well open with saline or vegetable purgatives but not with calomel. Sweating is desirable when the response is rapid and the heart is not too badly damaged. A slow response to sweating in hot air baths is often most exhausting. Venesection is of doubtful value except for the relief of cardiovascular complications. Lumbar puncture is often decidedly helpful especially in hypertensive nephritides with impending coma. Where the spinal fluid is under decidedly increased pressure, lumbar puncture should be repeated at two or three hour intervals.

The dyspnea or, more accurately, hyperpnea of uremia is due to a true acidosis but not, as in diabetes, the result of accumulation of abnormal acid bodies. Instead it is consequent on a lowering of the alkali reserve due to accumulation of acid phosphates. Where hyperpnea exists it should be corrected by the administration of sodium bicarbonate in doses up to one teaspoonful hourly. It is worthy of note that the acidosis of uremia is often even more severe than that of diabetes. The alveolar carbon dioxide tension sometimes reaches a figure as low as nine or ten.

We do not know precisely the cause of uremic twitchings and convulsions. There is however well authenticated evidence of a diminution of calcium in the blood paralleling the increase in phosphates. The blood calcium may be as low as 6 mg per 100 cc.

There is evidence therefore that the twitchings of uremia are associated with calcium deficiency and therefore analogous to tetany. The administration of calcium preferably in the form of calcium gluconate intramuscularly or intravenously for the treatment of uremia is therefore logical. It may be given in one gram doses hourly for three or four doses.

Coma associated with uremia is sometimes due to an actual cerebral edema, and when this is the case it may be combated with hypertonic sodium chloride intravenously in doses of 30 or 40 cc of 30 per cent solution. In many cases this has produced a return to consciousness.

In treating uremia where the case has not been studied previously, it is of the greatest importance to be certain that it is of renal origin. We should always bear in mind that obstruction of the urinary tract will produce a much faster increase in blood urea and nonprotein nitrogen than will nephritis. A kidney will still maintain a normal blood nitrogen content with 75 per cent of its structure destroyed. Complete *obstruction* however will produce rapid blood changes. It is therefore of importance to rule out pathology in the prostate or urinary tract, intestinal obstruction, and congestion of the kidneys due to cardiac failure.

EDEMA

Edema may be of cardiac origin in which the treatment with digitalization and one of the coronary dilating drugs, especially the purine basis, such as metaphyllin and theocalem, is obvious.

So called renal edema is met with both in pure lipoid nephrosis and in

glomerular nephritis. The renal changes in nephrosis appear to be secondary to basic pathology in the tissues and capillaries. The chief trouble in the kidneys consists in cholesterol deposits in the tubules. There is no evidence of inability on the part of the kidney to excrete nitrogenous substances. A diet rich in nitrogen is therefore desirable to compensate for the abnormal loss of albumin from the blood stream. It is in pure lipid nephrosis that the Epstein high nitrogen diet is indicated.

In addition urea in capsules in doses of from 15 to 100 grams daily promotes diuresis. Another essential feature of the Epstein treatment is the administration of thyroid extract. It should be given cautiously at first up to 5 grams daily and if no symptoms of hyperthyroidism result the dose may be increased and may at times be given in a quantity as great as 60 grams daily without untoward results. We should mention however parenthetically that there are observers who doubt that the low metabolic rate observed in nephrosis is a true observation. These believe that the increase in weight from water logging of the tissues gives a fictitiously low calculation.

According to Epstein the first indication of benefit from his program of treatment lies in a reduction of the blood cholesterol and frequent cholesterol determinations are therefore desirable. We would emphasize that pure nephrosis is very rare, that most cases diagnosed nephrosis are actually cases of nephritis with involvement of the glomeruli as well as the tubules and that in the late stages they develop hypertension and die of uremia. The high nitrogen and thyroid extract regime described above is only appropriate in true nephrosis.

In glomerular nephritis edema is frequent and even though hypertension and nitrogen retention may not be present they usually develop later. In this case a high nitrogen diet and the administration of urea must be employed with extreme caution if at all. Other means of combating the edema are preferable. Here the salt free diet should be applied. Food tables are readily available listing foods of low salt content and, without great trouble to the doctor or the patient, dietaries may be arranged which contain less than two grams of sodium chloride daily. Furthermore, fortunately such diets usually tend to produce an alkaline urine.

Diuretics are not indicated in the edema of glomerular nephritis. Their value lies in the treatment of cardiac edema with passive congestion of the kidneys, and of true nephrosis. Mercurial diuretics should be avoided in every form of nephritis. If the edema of glomerular nephritis does not gradually improve after removal of the original source of infection alkalization to render the urine barely alkaline, salt poor diet, and a reduced fluid intake, then the Osman diuretic treatment with alkali may be very cautiously instituted. Osman's stock mixture consists in 15 grams each of potassium citrate, potassium bicarbonate, sodium citrate and sodium bicarbonate in equal parts of peppermint water and chloroform water to make one ounce. This mixture is approximated in the preparation called citrocarbonate. The daily dose of alkali is 180 grams of total alkali, increasing by 60 to 100 grams daily until the urine is alkaline, with a hydrogen-ion concentration of 7.0 to 7.6. This will sometimes require a total dosage of 1,000 grams daily. The increase must be continued in spite of increasing edema—alkalinity of the urine being the one criterion of

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CLINICAL AND EXPERIMENTAL

BIOMETRICAL STUDIES OF HEAD LENGTHS OF HUMAN SPERMATOZOA*

By G. L. MORGAN, M.D., F.A.C.S., and HELEN HOIT, B.S., NEW YORK CITY, N. Y.

IN A RECENT article¹ we have dealt with the relationship of sperm morphology to fertility. However, not only the morphology of the spermatozoa and especially of their heads is important, but also the biometrics of these cells. Therefore, after the morphology of the semen had been determined, we proceeded to measure the sperm head length.**

Such studies have been made before, but were usually only concerned with the question of sperm dimorphism. Such dimorphism, however, we have found conspicuously absent. On the contrary, the closer the frequency polygon approached a normal frequency distribution, the better in most cases the reproductive fitness of the individual turned out to be, the coefficient of variability especially being that function of the frequency distribution of the population which formed an indicator of the fertility in the particular case. Thus the fertility decreased as the coefficient of variability increased. No semen specimen, of course, has sperms all of the same size, any more than all cells are ever morphologically perfect. At the same time we found that the sperm heads from one ejaculate did not vary beyond reasonable limits in normal cases.

Of the total number of 141 different cases in this present series, we were able to calibrate 124. Seven could not be calibrated because too few cells were present, or because the specimen spoiled after our morphologic count was made.

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**This was accomplished by projecting the image of the stained sperms onto a screen and measuring 300 or more head lengths (at a diameter of 3000 magnifications) with bow dividers.

and before we had time to measure the cells. Ten men of the series had no spermatozoa at all.

Table I* in the last three columns gives the simple function of the graphs obtained in the normal cases. It will be seen that only in a few instances does the standard deviation go above 1.5 and in only four cases is the coefficient of variation above .11, and in only two of these cases (Cases 37 and 41) above .15. In Case 41 it took two months for the woman to conceive so that a slight degree of impaired fertility may here be present, although we did not feel justified in excluding the case from the normal list. Case 37 was clinically absolutely normally fertile. The high coefficient here may however be due simply to random sampling as a few isolated large cells were found in this case. Fig 1 (Case 92) shows an even more marked example of this. Such isolated, markedly large or small cells may usually be found in any, even the most normal specimen, and while such cells are of course included in tabulating the morphologically abnor-

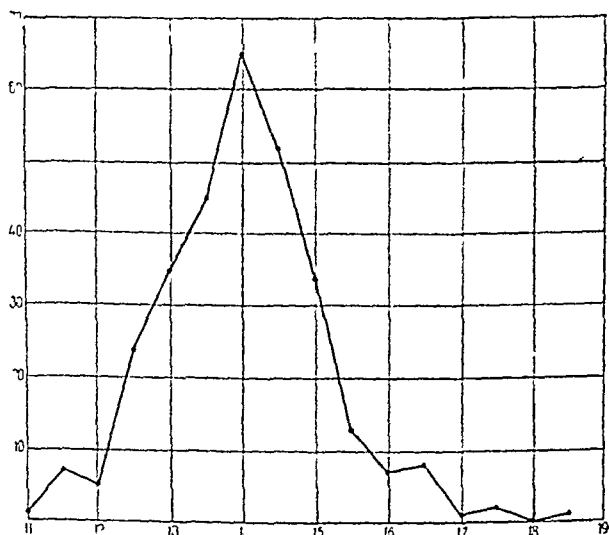


Fig 1—Case 92. Graph from a normal case showing several isolated large cells. Such cells do not form a true tail and must be rejected with reason when calculating the functions of the obtained curves.

mal forms, they really should be dropped in calculating the mathematical functions of the graphs, since they acquire a weight entirely out of proportion to their value, as their distance from the mean is squared and thus greatly increases the sum from which the standard deviation (which is one of the factors in determining the coefficient of variability) is calculated. One must, of course, be on his guard in rejecting any data obtained, and if a number of very large or small cells are found repeatedly in a semen specimen, these undoubtedly are significant as indicating a disturbance of spermatogenesis and must never be dropped. Such sperm cells may, in fact, constitute a true tail of the graph, whereas in the other cases we are dealing only with an apparent, or pseudotail.

*Table I may serve as an illustration of the way we tabulated our morphologic and biometrical results in the various groups of patients in this series. Tables for the other groups are omitted to save space. Only the results will be given here.

TABLE I
TABULATIONS OF OBSERVED CELL CHANGES PER 1000 IN 17 GENUINELY NORMAL CASES, AND THE SIMILAR CHANGES IN THESE SAME CASES GRAPHICALLY OBTAINED BY MEASURING 100 SPERM HEAD LENGTHS AT A MAGNIFICATION OF 7000 DIAMETERS

	O.C.S.F.	TOTAL ABNORMAL	IF ABN. ABNORMAL	ABNORMAL MINUS SIZE CHANGES	M*	P.P.	σ	P.E.	C	P.I.
1	92	361	193	58	12,988	± 0.072	1,727	± 0.077	10,217	± 0.281
2	301	111	111	21	11,117	± 0.053	1,708	± 0.079	10,173	± 0.80
3	15	172	132	27	11,117	± 0.011	1,119	± 0.031	8,923	± 0.28
4	65	233	144	15	13,500	± 0.051	1,311	± 0.078	10,008	± 0.78
5	18	121	133	59	11,672	± 0.051	1,383	± 0.078	9,426	± 0.60
6	10	271	170	68	11,670	± 0.017	1,215	± 0.073	8,067	± 0.27
7	68	160	91	28	11,755	± 0.017	1,091	± 0.030	7,095	± 0.16
8	17	102	161	70	11,810	± 0.050	1,283	± 0.075	10,801	± 0.20
9	81	131	111	27	12,878	± 0.016	1,151	± 0.077	9,185	± 0.23
10	11	211	172	78	12,608	± 0.058	1,183	± 0.031	11,751	± 0.32
11	57	191	112	61	11,517	± 0.016	1,187	± 0.077	8,752	± 0.21
12	18	172	181	76	11,745	± 0.059	1,556	± 0.041	11,081	± 0.30
13	16	261	181	71	11,748	± 0.057	1,177	± 0.041	11,501	± 0.26
14	27	271	161	66	11,915	± 0.053	1,166	± 0.077	9,717	± 0.28
15	71	162	136	66	11,915	± 0.018	1,212	± 0.031	9,211	± 0.31
16	91	111	112	17	11,668	± 0.018	1,151	± 0.016	11,721	± 0.31
17	50	263	162	61	11,612	± 0.055	1,151	± 0.016	11,721	± 0.31
18	55	231	131	70	11,673	± 0.018	1,212	± 0.031	8,165	± 0.27
19	91	231	162	75	12,518	± 0.011	1,052	± 0.029	8,107	± 0.10
20	2	292	162	61	11,528	± 0.056	1,111	± 0.010	10,722	± 0.63
21	28	270	151	61	11,700	± 0.052	1,279	± 0.015	9,613	± 0.26
22	6	201	151	65	11,777	± 0.015	1,170	± 0.032	5,153	± 0.27
23	61	182	161	65	11,828	± 0.067	1,266	± 0.015	11,710	± 0.24
24	17	191	171	75	11,921	± 0.051	1,389	± 0.038	10,646	± 0.29
25	51	263	171	11	12,162	± 0.018	1,299	± 0.031	9,700	± 0.20
26	59	139	122	11	11,687	± 0.051	1,297	± 0.036	9,357	± 0.26
27	16	262	131	30	11,913	± 0.011	1,151	± 0.031	8,067	± 0.20
28	21	251	101	51	11,225	± 0.042	1,052	± 0.030	8,100	± 0.23
29	111	221	112	50	12,753	± 0.049	1,052	± 0.027	7,823	± 0.15
30	52	91	61	11	11,755	± 0.042	0,998	± 0.077	1,139	± 0.25
31	102	176	125	50	11,100	± 0.052	1,111	± 0.035	1,606	± 0.21
32	111	123	123	37	11,137	± 0.019	1,212	± 0.031	9,007	± 0.23
33	112	357	163	70	15,157	± 0.058	1,501	± 0.011	9,009	± 0.218
34	118	112	91	70	11,320	± 0.017	1,200	± 0.031	9,009	± 0.218
35	119	291	151	35	12,507	± 0.041	1,117	± 0.071	8,923	± 0.15
36	125	131	181	56	11,925	± 0.011	1,105	± 0.030	9,115	± 0.27
37	128	372	182	80	11,728	± 0.051	1,160	± 0.037	9,871	± 0.21
Breeding record in cases 12 and 18 very good					Average				Average	
					13,171	± 0.050	1,271	± 0.075	9,511	± 0.50

* M is the mean, and gives the mean head length in mm at a magnification of 7000 diameters
 σ is the standard deviation and is the square of the average deviation of each cell
P.E. is the coefficient of variability, i.e., the standard deviation multiplied by 100 and divided by the mean
P.I. is the possible error

Tables II, III, IV and V of the original article (not reproduced here) show in their averages of the simple functions of the graphs no significant variation of the mean, relatively slight variations in the standard deviation, but most interesting differences in the respective coefficients of variability

In 11 couples of impaired fertility and clinically abnormal wife the coefficient of variability averaged 10.336 ± 0.287 . In 30 couples with impaired fertility but clinically normal woman the average was 12.252 ± 0.337 . In 29 sterile couples with clinically abnormal wife the figure is 10.557 ± 0.279 , whereas in 34 sterile couples with clinically normal wife the coefficient of variability rose

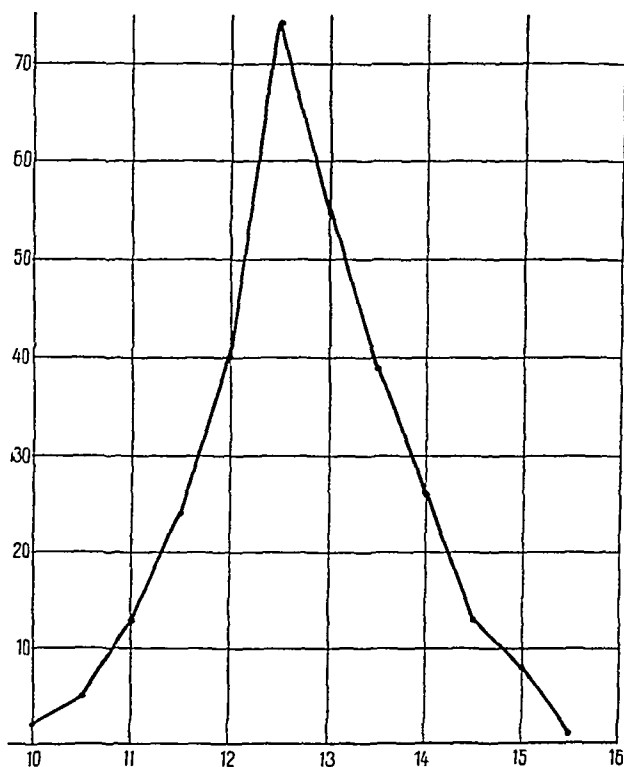


Fig. 2—Case 52 Graph from a man with a normal breeding record Coefficient of variation = 7.825 ± 0.215

to 13.082 ± 0.350 . Comparing these results with our normal groups and considering the unavoidable discrepancies which must arise in arranging all our cases only from the woman's side, the results show a very beautiful agreement. In our normal cases and those cases where the woman was abnormal, our coefficient of variability is hardly above 10.5, whereas in those cases where the woman was clinically normal, the figures are above 12 and 13.

The differences present in the graphs from presumably normal and abnormal men are made even more evident by Figs 2, 3 and 4. Fig 2 is from a normal man, Fig 3 from a man whose wife though clinically normal never conceived, and Fig 4 is from a man who had been married before and had four normal

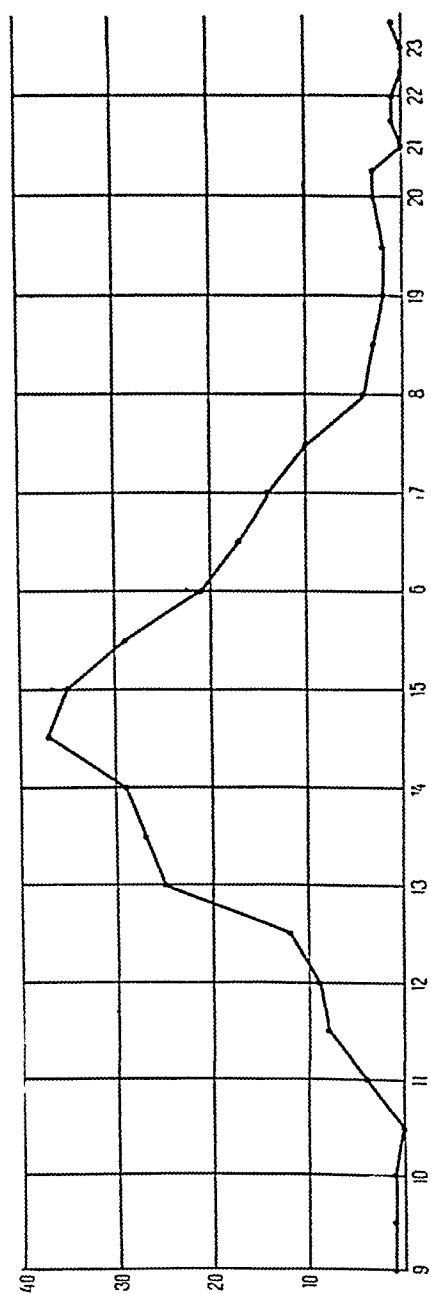


Fig. 7—Case 27. Graph from a man married three years to a normal woman without the wife conceiving although the couple desires children. Coefficient of variation = 11.509 ± 0.180

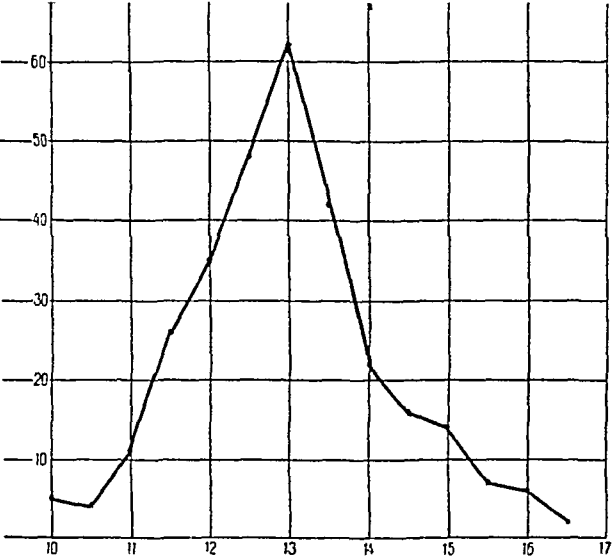


Fig 4—Case 105 Graph from a man married six years to a woman with marked ovarian deficiency. From a former marriage this man has four normal children the youngest seven years old. Coefficient of variation = 9.606 ± 0.265

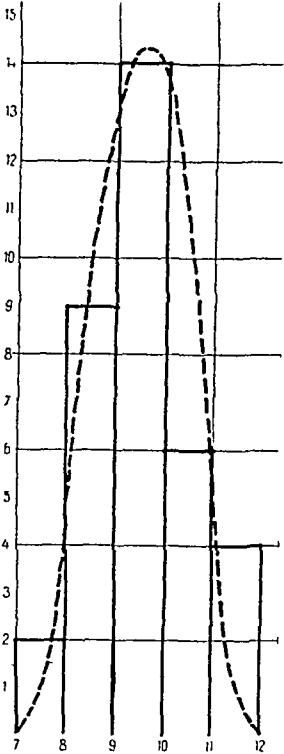


Fig 5—Histogram showing the distribution of the coefficients of variation of the sperm head lengths in the 35 normal cases

children from his first wife and was now married again six years without the woman having conceived. She was thirty years old, and had a definite ovarian dysfunction.

Since it is impractical to reproduce here more than a few illustrative graphs we have arranged the coefficients of variation of all our cases from the various groups in the form of histograms which seem to us very instructive. They are

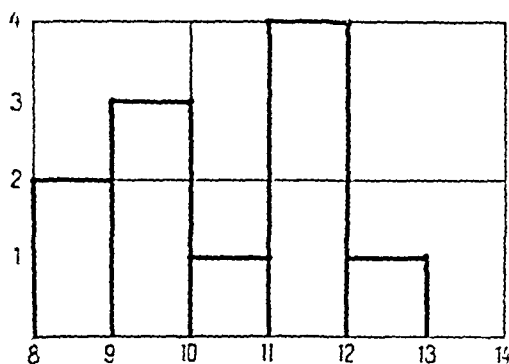


Fig. 6—Histogram showing the distribution of the coefficients of variation for sperm head length in 11 cases of impaired fertility where the woman was clinically abnormal.

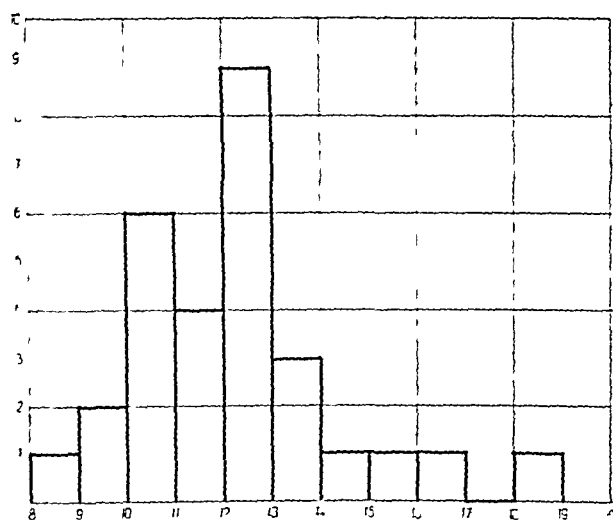


Fig. 7—Histogram showing the distribution of the coefficients of variation for sperm head length in 29 cases of impaired fertility where the woman was clinically normal.

arranged in class units of one, since this unit is two to four times the usual probable error, and thus mathematically of importance. In Fig. 5 from Table I, we have the 35 normal cases which were calibrated. We see at once that these cases group themselves in a fairly symmetrical almost normal or Gaussian curve with a mean lying between 9 and 10. In Fig. 6 we have too few cases to expect the normal frequency distribution, but we see that here again more cases are below 11 than above it, and only one case above 12. In this last case (Case 104)

the breeding record was difficult to determine, but there is strong evidence that the man was abnormal, as he had been married before and his first wife also had never become pregnant. In some of the cases between 11 and 12 outlying cells were present, besides in some of these the husband, as well as the wife, may have been of impaired fertility. In Fig 7 we see a decided difference from the two previous histograms. In the first place, although there are almost as many cases as in the normal group, there is no normal frequency distribution, and second, the weight of the graph lies above 12. At the same time, a number of the cases, 13 all told, fall below 12 and even below 11, and therefore within the normal limits. This is easily explained. While it is generally true that the biometrics run more or less parallel to the morphologic tables, this is not necessarily so. A cell may, for instance, be narrow and misshapen, and still be of normal length, and with many such cells present in a specimen, the calibration will give good

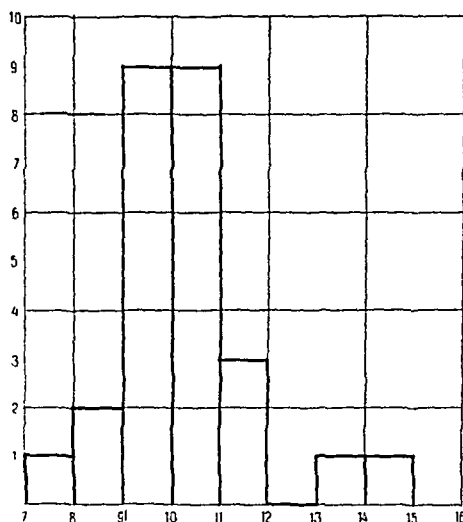


Fig 8—Histogram showing the distribution of the coefficients of variation for sperm head length in 26 cases of sterility where the woman was clinically abnormal. Note the relatively normal frequency distribution.

results. The coefficient of variation thus is of value only when it is beyond the normal limits. It might be thought of, therefore, that not only the cell head lengths should be calibrated, but also the width of the sperm head, but the question then arises where such width is to be measured. If we simply take the broadest part of the cell head, the important tapering forms which may be and often are of normal width anteriorly will escape, and if we do not take the broadest part of the cell, it is hard to designate any point at which the measurements are to be made. Carried to the extreme, one would have to make three dimensional measurements of each sperm head, which is of course out of the question. In general, the head length alone will be sufficient, since it must influence, except in rare and unusual cases, the volume of the cell head, and thus the amount of nuclear material present, and in a case where the biometrics really seem to fail, the morphologic examination is always available as an indicator. Thus in 7 of the 13 cases with a fairly low coefficient of variation the cytologic changes were marked. In five of the remaining cases abortions were a dominant feature,

and these may perhaps have been due to some undetected abnormality of the woman. The last case (Case 5) was also one of impaired fertility where sexual overloading may have played a role.

In Fig 8 where the men were presumably normal we again have a practically normal frequency distribution centering around 9 and 10 with only two cases beyond the normal limits and as stated above there is no reason why two abnormal or sterile people may not be mated to one another. In Fig 9 we have instead of the normal frequency distribution a decided and positive skew, and again as in Fig 7 the weight lies on the far side of 12 only seven cases being within the extreme upper limit of the normal. Case 12 (coefficient of variation between 8 and 9) will be taken up separately later. Of the remaining

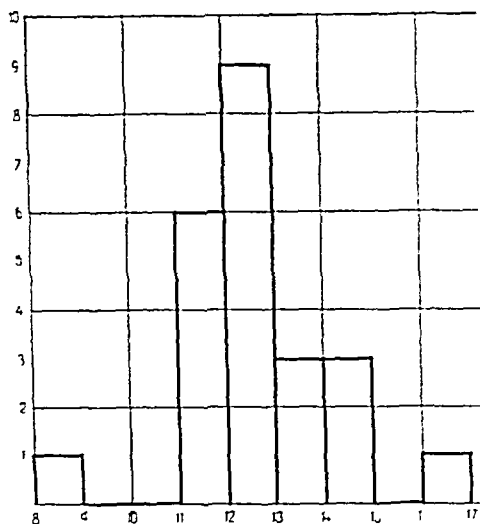


Fig 9.—Histogram showing the distribution of the coefficients of variation for sperm head length in 22 cases of sterility where the woman was clinically normal. Note the irregular frequency distribution and decided skew to the positive side.

six cases, five showed markedly abnormal cytology and the last one (Case 22) was apparently unexplained, although the head changes minus the size changes were higher than in the normal cases.

SKEWNESS AND TYPE OF CURVE

Aside from the simple functions of the graphs already given, there are a number of other functions which are significant in biometrics. One of these is skewness, or unequal distribution of the population, producing a lopsided curve. Fig 10 may serve as an example. Here we have a positive skew, tailing off in the direction of the larger ordinates. We have, therefore, picked out of the 12 normal cases whose graphs appeared most skew, and analyzed these graphs and have done the same thing with 12 of the abnormal cases, especially such as presented no explanation for the impaired or absent fertility, since both husband and wife appeared normal. Table II gives the complete mathematical analysis of the 12 normal cases and may serve as an example. It is to be noted, however, that the curve types are but rude approximations, since with such a small popu-

TABLE II
MATHEMATICAL ANALYSIS OF GRAPHS FROM TWELVE NORMAL CASES

CASE	B ₁	P E	B ₂	P F	A	P E	SAFENESS	P E	CURVE TYPE	REMARKS
27	0 116912	0 079438	3 350707	0 369131	0 257546	0 555221	+0 173857	0 048480	IV or VII	P Es only Approximated
41	0 106451	0 168221	4 452499	3 307564	0 032683	0 068924	-0 096308	0 081774	VII	
52	0 002209	0 001495	3 012722	0 195868	0 088093	0 032031	+0 023354	0 048013	IV	
40	0 000059	0 000075	3 778687	0 213800	0 000029	0 000046	+0 005263	0 070622	VII	
125	0 001061	0 000662	2 865412	0 156928	-0 002921	0 002259	+0 017954	0 017507	VII	
51	0 108978	0 210276	4 929641	5 841000	0 025010	0 130714	+0 087230	0 120325	I or VII	P Es only Approximated
37	0 078091	0 055684	3 236743	0 320866	0 249709	1 479720	+0 129769	0 017896	IV or VII	
134	0 018571	0 011487	2 888457	0 167851	-0 050174	0 116820	+0 075223	0 049474	VII	
50	0 008337	0 006659	3 287853	0 311131	0 011483	0 027258	-0 092670	0 049649	VII	
6	0 460093	0 428340	5 084301	4 750680	0 142220	0 412764	+0 200703	0 104700	I or VII	
92	0 111156	0 128502	4 044401	1 216486	0 049583	0 054127	+0 111255	0 075419	VII	P Es only Approximated
119	0 036399	0 021456	2 820904	0 157707	-0 053035	0 039968	+0 111363	0 047896	VII	

lation as 300 the probabilities of error frequently become so large that two or three different forms of Pearson's type curves could be fitted to the obtained data so that we simply had to choose that one which seemed most logical.⁴ Due to the carrying of a pseudotail produced by isolated cells in our calculations some of the graphs approach the heterotypic curves (Case 51 for example)

One thing however did stand out from our tables namely, that the normal cases showed more clearly normal curves (Type 7 of Pearson) than the abnormal cases

In Table III we present the skewness factors and the relation of this function to its probable error. According to Pearl the value of the skewness factor is unimportant unless it amounts to at least four times its probable error. This we see is not so in any of our normal men so that these can be considered disposed of immediately. Our abnormal cases however show four instances where the skewness factor is more than four times the probable error. In case 101

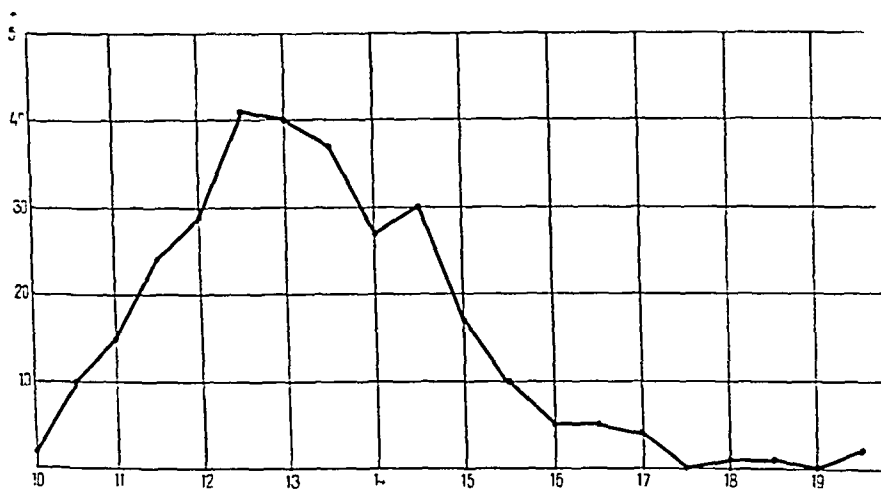


Fig. 10—Here we have a marked positive skew with a tailing off in the direction of the larger cells. The man had a very poor breeding record.

(Fig. 11) it is so slightly above 4 as hardly to be significant, and in addition, this curve is skew solely because of random sampling. We have on recalibration found smaller sized cells than the smallest in the present curve, but thought it interesting to show this figure in order to call attention to the possibility of artificially produced abnormalities. The remaining three graphs (not reproduced here), however, showed definite skewness. In two of these, Cases 32 and 109, the cytology of the semen was bad. In one of these two the coefficient of variation was also beyond the normal limits whereas in the other the only definite abnormality in the biometrics was the skewness. This case (Case 12) is, however, extremely interesting. This was one of our unexplained cases. The wife, married for the first time one and a half years ago, was normal except for a moderate retroversion which we did not deem a sufficient explanation for the existing sterility. The husband had been married nineteen years ago for the first time, and although no contraceptive had ever been used his wife only had had one

TABLE III

TABLE SHOWING SKEWNESS AND RELATION TO PROBABLE ERROR OF THIS FUNCTION IN EACH CASE OF 12 NORMAL AND 12 ABNORMAL CASES OF TABLES XI AND XII

NORMAL CASES			ABNORMAL CASES		
CASE NO	SKEWNESS	V P F	CASE NO	SKEWNESS	V P F
27	+0.153857	3.23	44	-0.000175	0.003
41	-0.096308	1.18	124	+0.014297	0.30
52	+0.023354	0.49	138	+0.069322	1.46
40	+0.005263	0.10	109	+0.745100	6.03
125	-0.017954	0.38	67	-0.108474	1.30
51	+0.087290	0.73	99	+0.598295	3.04
37	+0.129769	2.71	12	+0.297601	5.62
134	+0.075223	1.52	32	+0.293362	4.58
50	-0.032670	0.66	101	+0.192488	4.08
6	+0.200703	1.03	107	+0.380051	3.95
92	+0.111255	2.01	24	-0.127331	2.62
119	+0.111363	2.33	30	-0.118704	2.26

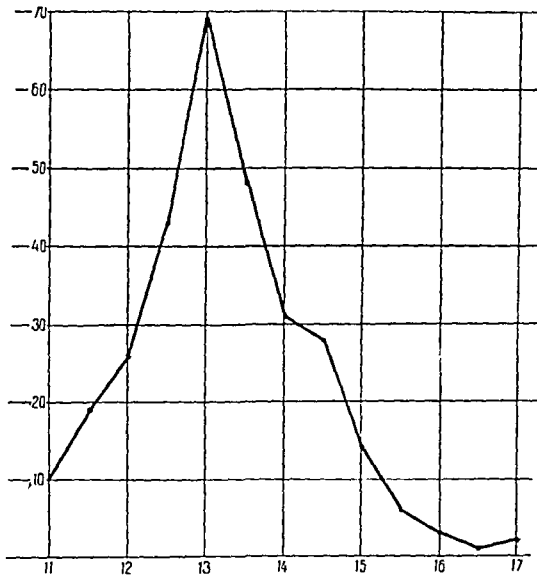


Fig. 11—Case 101. Graph from a man married to an abnormal woman. Skewness factor only 4.08 times the probable error. Seminal morphology good. Coefficient of variation 8.475 \pm 0.237. Skewness due to random sampling; see text.

abortion of two months one year after marriage, and a normal child five years after marriage. In 1915 this man was separated from his first wife and married the present one. The semen examination showed a normal morphology and a (normal) coefficient of variation of only 8.5, so that we were at a loss to explain this case until our complete mathematical analysis revealed the high skewness factor. It may perhaps be doubted that such a delicate factor as skewness, the result solely of advanced mathematical analysis of the frequency distribution polygon, can be significant. At the same time it must be remembered that skewness can only be produced on the negative side by an unusual number of under-sized heads, and on the positive side by a great number of abnormally big cells,

and since no significant distortion due to the presence of such sperm heads is found in the semen of normal cases the condition is abnormal

CORRELATION OF LABORATORY AND CLINICAL DATA

In investigating the clinical histories of our patients after the semen examination was completed it was really astounding to see how closely in the great majority of cases we could predict the clinical breeding record of the case from our microscopic findings except of course in those cases where we considered the husband normal but the couple had a bad breeding record due to some pathology of the wife. In thus looking up the clinical history after we had completed our laboratory investigations we hoped to exclude all subjectivity in the interpretation of our findings. We will however not deny that a certain amount of subjectivity may have entered into the interpretation of the clinical histories. At the same time we tried as much as possible to avoid this and to judge as impartially as we could whether or not the minor lesions which the woman at times presented could possibly explain the abnormalities of fertility found in the couple. We do not at all deny that in thus judging our cases we may not have erred in some. At the same time seminal micropathology and clinical breeding record agreed so closely in the vast majority of our cases that we can hardly consider the possibility that such agreement was the result only of misinterpretation of the clinical records. Besides such misinterpretation is really possible only in doubtful cases and hardly in those where the couple had normal children in rapid succession or had none at all.

Because we wanted to avoid the subjective influence as much as possible all special examinations on the women were also deferred until the semen was examined. Naturally in so doing we at times had disappointments inasmuch as a man whom we considered of greatly impaired fertility was found to be mated to a woman who had closed tubes. Thus our whole work on this case would become useless as the man's fertility was not determinable. Such results are however to be expected from time to time.

In most cases the morphologic changes and the coefficient of variation ran more or less parallel but it also occurred that the specimen showed a poor morphologic picture and gave a good biometric result. This has already been explained. On the other hand due to small graded size changes, unappreciable under the microscope under the magnification employed in tabulating sperm morphology an apparently good cytology may be present whereas the biometrics show up marked size differences and result in a high coefficient of variation. A normal cytologic or biometric result by itself therefore means nothing but an abnormal one in either case becomes significant.

In doubtful cases it is of value to count the abnormal sperm heads minus their size changes as large numbers of narrow tapering or misshapen cells are very important. When the figures for this last count go much above 80 or 85 per 1000 the man is at least to be suspected to be of impaired fertility. In Case 76, for instance the woman was normal, but did not conceive for a number of months despite normal sex relations. In this case the head changes approached 200 per thousand, the head changes less the size changes were 85 per thousand

and the biometrics, while well within normal limits, were at least above the average

It seemed to us that those cases where both the morphology of the semen and the biometrics gave moderately high figures were of lower fertility than those where only one of these two means of examination gave equally high results

For our studies patients who had been married before were especially desirable. We had, however, only eight such cases (Cases 7, 11, 12, 49, 54, 77, 104, and 105). Of these cases, Case 12 has been discussed under skewness and Case 11 (sperm motility lost after an attack of influenza) will be taken up later. Case 54 is inconclusive since both husband and wife were abnormal. In Case 7 the woman had normal children from a previous marriage, and now was apparently sterile. The semen was poor according to our standards. In Case 49 the woman had been pregnant in a previous and the present marriage, and now had closed tubes, apparently after an abortion. The husband was normal. In Case 77 the woman now married without becoming pregnant, was normal and had been pregnant in a previous marriage. According to our standards the second husband was infertile. Case 104 has already been discussed. Case 105 is shown in Fig 4 and its legend.

As mentioned in the discussion on biometrics there were among the total number of our patients some in whom we could offer no explanation for the infertility. This is only to be expected. In fact, we would have been extremely suspicious of our methods of investigation had all the cases followed the rule without deviation. Among the 141 married couples examined by us there were 104 (141 total number less the 37 normal) of impaired fertility and sterility. Counting all, even the partially unexplained instances of impaired fertility, we had only eight couples in whom no assignable reason for the disturbed fertility could be discovered. In all of these the wife was apparently normal. It is true that in four of these eight cases we did not examine the woman, but she was reported to be normal. In one of the eight we really had a rather high cell count and biometrics so that this case may perhaps be partially explained. In two others sexual overloading was present, but the semen showed only changes well within normal limits. One case (Case 20) showed a condition which we believe is not infrequently met with. This woman after an apparently traumatic first miscarriage had other spontaneous miscarriages, all about the same time as this one. We can offer no explanation whatsoever for this.

It is we think exceedingly interesting that of our unexplained cases seven were in the group of impaired fertility with abortions as a prominent feature, and only one (Case 22, already referred to) in the sterile group. To us it indicates that the aberrations of the external indicators of normal ovulation (menstruation vaginal flora etc.), when of sufficient degree to cause sterility in the female, are generally easily recognizable.

Although thus among our 21 couples in whom spontaneous abortions occurred, there were six (Cases 5, 20, 44, 67, 82, 138) where no explanation could be elicited including Case 67 with seven, and Case 20 with five spontaneous abortions it is not without weight that the sperm head abnormalities in this group averaged 200 per thousand while the coefficient of variation was 11.571, whereas in the intermediate group, where the woman was abnormal, the average

number of sperm head abnormalities per thousand was only 158 and the coefficient of variation but 10.125. This would certainly seem to indicate a participation on the part of the male in the production of these interruptions of gestation. Such participation can naturally be only of the nature of germ plasma defects and raises the question asked by one of us (M.) before whether the products of gestation in such cases are not inherently abnormal and abortions only an attempt on the part of Nature to prevent abnormal offspring. Trying to prevent abortions due to these causes thus becomes a procedure of doubtful value and stresses the fact that before attempting to treat cases of habitual abortion we must avail ourselves of every possible method to determine the underlying etiology including a careful examination of the husband. Hydatid mole and other abnormalities of the gestational products considered in this light also take on a different aspect but unfortunately with the exception of Case 95 (with repeated fetal abnormalities and a semen showing 12 per cent double sperm forms) we had none of the above mentioned gestational abnormalities in our present series. It is of interest we believe to state that Case 95 had a cytology just beyond our assumed normal limits and a normal biometrical status. In no other case however did we find any such number of double forms as were present here. Many specimens of course showed double forms but these never exceeded 1/2 or at the outside 3 per cent of the total number of sperms counted.

Of especial interest to us were those cases where apparently the clinical history did not agree with our laboratory findings and it is worth while to study a few of those cases here.

In Case 11 I obtained the history that the man had been married before and had two normal children and now was married again without the wife conceiving. As I could not at that time examine the wife I naturally thought from the history I received that the woman was at fault. The seminal micropathology was however poor and I found out later that the wife was perfectly normal and had conceived quickly and easily from three or four different men.

Case 74 is another interesting one. Here the man according to our findings was of impaired fertility yet the woman claimed she always conceived easily but that contraceptives (coitus interruptus) had been practiced for years. On being advised against this procedure she became "more or less careless" according to her own statements but so far in six months had not conceived.

Case 75 is an exact duplicate of Case 74 with exactly the same outcome. In addition, the husband had gained eighty pounds since marriage and now was definitely obese. While it is true that Kisch⁸ and also Mettenleiter⁹ claim that obesity causes infertility in the female, but not in the male other authors as Dickenson and Cary¹⁰ do not agree with this and report cases where the semen improved considerably on reducing the man to normal weight.

In Case 99 we had another instance similar to the last two, but with a very different outcome. Here too, the woman claimed she conceived easily when contraceptives (coitus interruptus) were not used but we thought the man to be of a degree of impaired fertility which according to our standards should at least make impregnation difficult. As it happened the woman in the ten days following her next menstrual period for one reason or other had natural intercourse without using any contraceptive and did not become pregnant. She

thereupon became careless but did not conceive until three months later, the pregnancy ending in a spontaneous abortion. The woman was normal except for the fact that she had a moderate retroversion which we did not consider an explanation of this case.

In Case 96 we seemed at first sight to have an instance which was contrary to our theories, since the seminal micropathology was poor, but the woman had had a child only three months before. We learned from the wife, however, that shortly after she became pregnant her husband had fallen off a ladder, had been in the hospital for months, and still was in very poor physical condition.

In Cases 103 and 108 the sperm abnormalities and the biometrics ran to high figures, although both women had a number of young children, but in each case the husband was in poor physical shape.

In Case 137, however, where the woman was three months pregnant when we examined the husband's semen, no history of physical disability of the man could be elicited and still the figures of the seminal examinations were higher than in any other case where the woman had recently been pregnant. However, the breeding record in this case was rather in doubt as the woman had conceived three times, though coitus interruptus was practiced so that it is difficult to say how much of each sterile interval had been due to the contraceptive method employed.

At the same time, it is perfectly possible for a woman married to a man of impaired fertility to happen to conceive on sufficiently often enough repeated intercourse. In another exactly similar case the woman may, however, not happen to become pregnant, and thus in human beings, cases of impaired fertility and lost fertility may offer difficulties of classification as discussed under the heading of the determination of the breeding record.¹¹ Of course, one can always say that the husband is not the only man living but I do not believe such an easy explanation to be allowable unless absolute proof of it exists, and there were no indications whatsoever pointing in this direction in this last case. It is of importance here to mention that Williams and Savage¹² have at times found a poor semen in an apparently good breeding bull, but in every such case breeding troubles soon appeared, the disturbance of fertility thus being morphologically discernible in the semen before it became clinically evident.

These eight cases just discussed were about the only ones in our series where at first it appeared as though a poor semen specimen were linked to a clinically good breeding record.

It is striking that in a large proportion of these marriages coitus interruptus was practiced. It has long been recognized even by the laity that coitus interruptus has a deleterious effect, but this was supposed to be mainly on the nervous system. Here, however, we have an indication that this procedure, perhaps due to the frequently concomitant unrelieved congestion, may actually affect spermatogenesis.

We had a total of ten cases (Cases 47, 70, 74, 75, 91, 96, 99, 102, 131, and 137) in whom admittedly coitus interruptus was more or less persistently practiced. In Case 91 this method of contraception had been used for about two years. It was practiced on and off in Case 102, in moderation in Cases 47 and 99, and for a fairly long number of years in Cases 131 and 137, and excessively in Cases 70, 74, and 75.

On looking up the seminal micropathology of these ten patients we found it normal in Cases 91 and 102 poor in 47 around the limits of the normal in 99 poor in 131 rather poor in 137 and very poor in Cases 70 74 and 75. While these few cases allow of no conclusions the findings are at least suggestive and should be followed up. Undoubtedly individual variations will be found here too.

REEXAMINATIONS

Although we made many reexaminations both on the same and different samples of the same semen such examinations were mostly for our own information and on semen specimens obtained within a short time of one another. Seven of our abnormal cases however returned after three or four months for a re-examination. Their general and sexual habits had been investigated at the time of their first examination and in every case the defects present (especially lack of exercise etc. resulting in poor general physical health) had been advised about and rules given to the patient to help him get into better physical trim. Vitamin (especially B, C and E) containing foods were advised though generally a deficiency of diet was not to be elicited in these patients. In every case but one (Case 32) where the man admitted not having been able to carry out the instructions very well the patient at the time of reexamination was both subjectively and objectively in decidedly better physical shape than before and even Case 32 was somewhat improved.

Table IV shows the results of the various semen examinations made on these seven patients. In every one of these men the semen examination gave figures very much beyond our assumed normal limits when they first presented them-

TABLE IV
REEXAMINATION OF THE SEMEN OF SEVEN MEN OF DECREASED OR ABSENT FERTILITY

CASE NO	TOTAL ABNORMAL	HEADS ABNORMAL	M	I F	σ	P F	C	P F
10 I	601	273	15 120	± 0.075	1 928	± 0.073	12 751	± 0.251
10 II	503	252	14 767	± 0.066	1 684	± 0.046	11 404	± 0.314
11 I	292	262	12 494	± 0.064	1 644	± 0.045	13 158	± 0.362
11 II	373	231	12 954	± 0.059	1 522	± 0.042	11 749	± 0.324
11 III	314	143	13 398	± 0.054	1 397	± 0.038	10 427	± 0.287
90 I	354	243	13 465	± 0.063	1 624	± 0.045	12 061	± 0.332
90 II	353	251	13 545	± 0.056	1 450	± 0.040	10 705	± 0.398
32 I	514	261	13 615	± 0.064	1 632	± 0.045	11 987	± 0.330
32 II	394	223	13 250	± 0.062	1 591	± 0.044	12 008	± 0.331
23 I	511	364	14 838	± 0.080	2 049	± 0.056	13 809	± 0.380
23 II	423	244	15 150	± 0.068	1 742	± 0.048	11 495	± 0.317
78 I	360	244	15 905	± 0.072	1 837	± 0.051	11 550	± 0.318
78 II	354	271	15 472	± 0.061	1 564	± 0.043	10 109	± 0.278
85 I	413	302	16 020	± 0.077	1 984	± 0.055	12 385	± 0.341
85 II	502	304	15 890	± 0.071	1 811	± 0.050	11 397	± 0.314

selves so that thus far we have not been able to bring any of them down to normal with the exception of Case 11, and here, unfortunately after an attack of influenza sperm motility was lost

Nevertheless, three of the men (Cases 10, 11 and 93) showed improvement both in the morphology and biometrics of the semen, and two others (Cases 90 and 85), an improvement in the biometrical figures although the morphology remained about the same. In the sixth man the morphology was improved, but the biometrical result about the same as at the first examination. The last patient (Case 79) showed more abnormal sperm heads than before, but a somewhat lower coefficient of variation.

Another fact worth pointing out is that Case 11 had many small sperms which gradually increased decidedly in size with improved physical health, whereas Cases 10 and 78 had large cells which decreased somewhat in size, as shown by the various means. The last change, however was not so decided as the first and was approached in value in the opposite direction by Case 23.

SUMMARY

If we summarize here our work on impaired human fertility reported in this and previous articles including about 60 cases not yet published we would say:

1 Sterility and fertility are not separate and opposed entities. Fertility is of varying degrees and starting from the normal, proceeds gradually downward to such low values that clinically sterility is present. Absolute sterility is, however, much less frequent than commonly supposed and being due usually to rather gross lesions is easily determinable in most instances. In the exact determination of the fertility of any given individual the anamnesis, general, menstrual, sexual, etc. is almost as important as the physical examination. Especial attention must be paid to the practice of coitus interruptus as it would seem as if this method of contraception detrimentally affects spermatogenesis.

2 In the female the external indicators of normal ovulation (normal ovaries on palpation, normal menstrual cycle, normal relation between the histologic picture of the endometrium and the menstrual periods and a normal [bacillary] flora in a vagina undisturbed for at least four to five days) seem to be sufficiently reliable to allow of the determination of impaired fertility of sufficient degree to cause sterility. Lesser degrees of impaired fertility, resulting in miscarriages and premature labors seem, however, to be harder to determine and may at times escape detection by the methods thus far employed by us. These cases are, however, being investigated along other lines.

3 Sexual incompatibility from one source or another must at least, judging from the small number of unexplained cases of disturbed fertility in the present series of cases, be considered rare.

4 By means of a careful and exhaustive examination of the semen, it seems possible to determine not only the fertility but also the degree of fertility of any given man. Such a semen examination must include besides other factors a careful consideration of the number of spermatozoa present, their motility, their morphology and the biometrics of the sperm head lengths. Both the number and the motility of the spermatozoa present in any sample of semen must be judged very guardedly and investigated thoroughly as purely temporary or

accidental external factors at times even of a trivial nature may give rise to misinterpretations.

Judging from our present small series of cases the morphology of the spermatozoon especially of the heads seems to be the best and most reliable indicator of the fertilizing power of these cells. Furthermore the relative number of abnormal heads emitted apparently gives a direct index of the reproductive fitness of the individual. Thus we found no man in our series with more than 19 to 20 per cent abnormal heads who had a good breeding record. In doubtful and borderline cases it is of value also to count the head changes minus the size changes as narrow and tapering forms are of especially sinister import and in every case where such a count rose materially above 7.5 to 8.5 per cent disturbed fertility was present.

The biometrical results from measuring the sperm head lengths never gave any evidence of sperm dimorphism. The graphs in the normal cases always approached closely the normal type of curve.

Of the simple functions of the obtained curves the coefficient of variation is the most important and was seldom much above 11.0 in a normally fertile man. The upper physiologic limit of this function seems to be around 11.5.

In every case where abnormal curves and coefficients of variation above the normal limits or a mathematically significant skewness was present the man's breeding record was poor.

In most cases the morphology of the semen and the biometrical results ran parallel. In some instances however only the morphology was bad and in others only the biometrics. Thus neither a normal morphology nor a normal curve alone mean normal fertility whereas an abnormal finding in either signifies a disturbance of spermatogenesis and hence of fertility.

From our figures it would seem as though lesser degrees of disturbed spermatogenesis allow conception to occur but tend to premature interruption of the pregnancy but when the fertility sinks as low as six tenths of the normal value clinical sterility usually results.

5 The described method of semen examinations is at present only of diagnostic import. It is evident however since disturbed spermatogenesis is only a symptom and careful search must be made in each case for the underlying cause and this treated that repeated determinations of the cytology and the biometrics of the sperm head lengths will allow of an accurate estimation of the therapeutic results obtained. In the present series of cases we have found spermatogenesis to be most favorably influenced by sexual rest and improvement of the general physical status of the patient.

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THE EFFECTS ON PNEUMOCOCCI OF SODIUM DEHYDROCHOLATE, A BILE SALT DERIVATIVE III*

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INVESTIGATION of the relations of the bile salts to pneumococci appears to be promising of practical therapeutic and prophylactic results. The number of papers dealing with this subject is increasing.

This paper represents a continuation of work already published^{1,2} and deals with the effects of sodium dehydrocholate on tissue, on pneumococci, and on pneumococcus infection.

The chemical nature of sodium dehydrocholate was given in a previous paper.² It was first made by Hammarsten³ in 1881 and was synthesized by Wieland⁴ in 1927. The sodium dehydrocholate used in this work was obtained from the manufacturer in a 20 per cent aqueous solution in sterile ampules.

REVIEW OF THE LITERATURE

Rigobello⁵ has recently investigated the behavior of the bile acids in the lysis of pneumococci and Downie, Stent, and White⁶ have recently published an important paper. In this paper it was reported that pneumococci were soluble in all bile salts except sodium dehydrocholate and sodium dehydrodeoxycholate. This is apparently at variance with other work.² However, in this latter work the pneumococci used were grown in animals and used without culturing on artificial media. Downie, Stent, and White in their work used twenty-five strains of pneumococci of different types. Sixteen of these were grown for long periods on artificial media. Nine strains were more recently isolated but were nevertheless grown on artificial media. For their tests, cultures in hormone broth were used, 0.9 cc. of culture being used with 0.1 cc. of bile salt solution. The sodium dehydrocholate used by Downie, Stent, and White was prepared according to Hammarsten.³ Considering the great difference in the methods employed, it is not surprising that the results are different. The chemistry of pneumococci grown on artificial media is apparently different from that of those grown in animals.

The English investigators⁷ reported a rough Type II pneumococcus resistant to solution in the bile salts. Elton⁸ refers to reports that strains of pneumococci have been encountered which were resistant to solution in bile salts. Several hundred strains of pneumococci isolated at the Rockefeller Institute were all reported to be bile soluble.⁹

The work of Downie, Stent, and White is admirable from the standpoint of what takes place in the test tube between various bile salts and relatively avirulent artificially grown pneumococci. From a utilitarian standpoint the toxicity of the bile salts must be given great weight. These workers have shown that

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¹The work of this paper was performed during a postgraduate course in pathology and bacteriology of the U. S. Veterans Bureau in affiliation with the Army Medical School.

sodium deoxycholate has the greatest pneumolytic power but this salt is also quite toxic. The least toxic of the bile salts, sodium dehydrocholate, does not produce lysis of artificially grown pneumococci as shown by these workers and also by the work of this paper. Nevertheless, it produced lysis of virulent pneumococci taken directly from the animal body in the presence of body fluid.²

Baijot¹⁰ has treated persons of the black race suffering from pneumonia, with bile salts intravenously. The preparation which he used was a 7 per cent solution of sodium taurocholate containing 2 per cent of magnesium sulphate. The reason for adding the magnesium sulphate was not given. This worker did not report any local or general toxic effects except a hemoglobinuria when his preparation was injected too rapidly. The color of his patients probably obscured the local injury to the veins. He treated four colored men, three of whom recovered and one died. The one who died had consolidated lungs but cultures for pneumococci were negative. A rabbit injected with blood from this case survived. Baijot points out that four out of seven of his patients treated in other ways died and that the mortality from pneumonia is very high in the colored race, being about 70 per cent.

In the work of Ross¹¹ where rabbits were fed Pneumocholin (bile salt dissolved pneumococci) mixed with their food, a high degree of immunity was produced. There was also produced considerable immunity with the same method using hydrochloric acid treated pneumococci and macerated organisms. It must be pointed out that these latter two preparations of pneumococci became bile treated when they reached the rabbit's intestine. The simplicity of Ross' method suggests a direct human application. Indeed, the mere swallowing of capsules containing pneumococci or pneumocholin may produce an immunity of practical importance. It may be that the same procedure could also be used in the early treatment of pneumonia to build up an immunity before any natural immunity appears.

EXPERIMENTAL WORK

In the following experiments pneumococci of Type I only were used. They were recently isolated organisms, but were transferred several times on blood agar and in calcium carbonate broth. They were agglutinated by Type I serum. They were soluble in sodium taurocholate but were insoluble in sodium dehydrocholate. The rabbits used were fairly uniform in size and weighed between 2.0 and 2.5 kg.

The sodium dehydrocholate was always administered in a 10 per cent concentration in 0.85 per cent sodium chloride solution, it was prepared by mixing equal volumes of a 20 per cent aqueous solution of sodium dehydrocholate and a 1.7 per cent solution of sodium chloride. In the following experiments where sodium dehydrocholate was used in 10 per cent concentrations in isotonic salt solution, it was prepared in this way. As the drug was obtained in a sterile condition it was only necessary to mix it with sterilized salt solution and to handle it under aseptic conditions.

EXPERIMENT I (TABLE I)

Intraperitoneal Injections of Drug and Organisms—This experiment was devised in order to obtain an estimation of the degree of the antipneumococcus action of sodium dehydrocholate *in vivo*. Pneumococci were injected intraperi-

tionally in rabbits and then at different intervals of time were followed by injections of sodium dehydrocholate. In one case the drug was given before the organisms and in another case the organisms and drug were given simultaneously.

All injections were made intraperitoneally. Rabbit 1 was given 1.0 gm (0.4 of a calculated fatal dose) of sodium dehydrocholate (10 per cent concentration in isotonic salt solution) as a control and survived without becoming sick. Rabbits 2 and 3 being positive controls were given 0.5 cc and 1.0 cc respectively of a 24 hour broth culture of pneumococci. The remainder of the rabbits were given 1 cc of the same culture. At varying intervals of time after the injection of the pneumococcus culture the test rabbits were given one dose treatments with sodium dehydrocholate. Rabbit 4 received the drug thirty minutes before the organisms. Rabbit 11 received the pneumococci plus the drug in the same syringe. The organisms were thus exposed to the drug less than one minute in this instance.

TABLE I
INTRAPERITONEAL INJECTION
ANTIPNEUMOCOCCUS ACTION OF SODIUM DEHYDROCHOLATE

RABBIT	INFUOCOCCUS CULTURE	10% SODIUM * DEHYDROCHOLATE	TIME IN MINUTES	RESULT DAYS
1 C	0.0 cc	10.0 cc	—	S
2 C	0.5	0.0	—	D 1
3 C	1.0	0.0	—	D 1
4	1.0	5.0	30	D 2
5	1.0	5.0	60	D 3
6	1.0	5.0	150	D 4
7	1.0	10.0	30	D 4
8	1.0	5.0	30	D 4
9	1.0	5.0	5	D 5
11	1.0*	5.0	0	S

D=died S=survived

*Drug and organisms given together

**In isotonic salt solution

Although the pneumococci were not dissolved on being exposed to sodium dehydrocholate they did not grow on blood agar and they did not kill Rabbit 11, nor make him sick. The sooner the sodium dehydrocholate was administered after the pneumococci the longer the test animals lived.

EXPERIMENT I-A

Effect of Sodium Dehydrocholate on Veins and Peritoneum—The following experiments were performed to determine the effect of aqueous and of isotonic solutions of sodium dehydrocholate on veins and peritoneum.

Rabbits 10 and 12 were given injections intravenously of sodium dehydrocholate in 20 per cent aqueous solution. This solution could be injected only with great difficulty as it caused the animals discomfort with consequent struggling.

These rabbits were also given intravenous injections of sodium dehydrocholate 10 per cent in 0.85 per cent sodium chloride solution. This mixture could be given as readily as isotonic salt solution alone with no discomfort to the animals.

Rabbits 10 and 12 were each given intraperitoneally 0.5 gm of sodium dehydrocholate 10 per cent in 0.85 per cent sodium chloride solution. Autopsy of these animals after several days revealed no evidence of peritoneal injury. There was a slightly increased amount of peritoneal fluid, but no adhesions, fibrinous exudate or other evidence of peritoneal injury.

EXPERIMENT II (TABLE II)

Intravenous Injections of Drug and Organisms—Since the results of Experiment I were encouraging a similar experiment was undertaken making all injections in the rabbits' ear veins. The sodium dehydrocholate was given in a 10 per cent concentration in isotonic salt solution. A 24-hour broth culture of pneumococci was used. In this experiment four daily injections of the drug were given while only one dose was given in Experiment I. The first dose of the sodium dehydrocholate was given on the same day that the culture of pneumococci was given. The time interval is given in Table II. The controls died in approximately one day. Rabbit 16 died in one day, its back was accidentally broken. One rabbit survived, one lived approximately four times as long as the controls and another six times as long.

Rabbit 1, used as a control for the drug in Experiment I was again used in this experiment. Ten minutes after receiving a fatal injection of pneumococci it was given 1 cc of 10 per cent sodium dehydrocholate in 0.85 per cent sodium chloride solution and 0.5 cc of pneumococcus culture in the same syringe. No further treatment was given. This rabbit survived.

Rabbit 11, a survivor from Experiment I, was included in this experiment and was given a fatal dose of pneumococci but no drug. It was injected on Feb 19, 1931, intraperitoneally with a mixture of pneumococcus culture and 10 per cent sodium dehydrocholate. On Mar 10, 1931 nineteen days later, it received in Experiment II a fatal injection of pneumococci but survived without becoming sick.

TABLE II
INTRAVENOUS INJECTIONS
ANTI PNEUMOCOCCUS ACTION OF SODIUM DEHYDROCHOLATE

RABBIT	3 10-31 PNEUMOCOCCI DOSE BROTH	SODIUM DEHYDROCHOLATE 10 PER CENT**					RESULT
		INTERVAL	3-10-31	3-11-31	3-12-31	3-13-31	
14 C	0.2 cc	—	0	0	0	0	D 32 hr
15 C	0.4	—	0	0	0	0	D 20 hr
16	0.4	10 min	2 cc	0	0	0	D Acc
17	0.4	15 min	1 cc	2 cc	1 cc	1 cc	S
18	0.4	3 min	1 cc	2 cc	1 cc	1 cc	D 4
19	0.4	10 min	2 cc	2 cc	1 cc	1 cc	D 6
1	0.4	10 min	*1.5 cc	—	—	—	S
11	0.4	—	—	—	—	—	S

*1.0 cc 10 per cent sodium dehydrocholate + 0.5 cc pneumococcus culture
D=died S=survived C=control Acc=accidentally
**In isotonic salt solution

On autopsy of Rabbit 18 there was recovered from the heart's blood a growth of *B. coli* but no pneumococci. Pneumococci were recovered from Rabbit 19 in pure culture.

EXPERIMENT II A

Subcutaneous Injection of Sodium Dehydrocholate—In order to determine the effect of subcutaneous injection of sodium dehydrocholate a 10 per cent concentration of this drug in 0.85 per cent NaCl solution was used. Rabbits 1, 11, 17, 20 and two guinea pigs were injected subcutaneously after clipping the hair with amounts varying from 1 to 5 cc. After twenty-four hours there was no evidence of any tissue damage.

EXPERIMENT III (TABLE III)

Immunity Tests—The results of Experiments I and II indicated that there was some immunity produced against pneumococci by a mixture of these organisms and sodium dehydrocholate. In this series Rabbits 1, 11 and 17 received immunizing injections as shown in Experiments I and II and survived. They were used again in this experiment to see if their immunity continued.

Rabbits 24, 25, 27 and 30 received injections of an antigen prepared by mixing equal amounts of an aqueous solution of 20 per cent sodium dehydrocholate and a 24 hour pneumococcus broth culture just before using. It is proposed

TABLE III
INCREASED RESISTANCE TO PNEUMOCOCCI DUE TO PNEUMOCHOLIN-D

RABBIT	PNEUMOCHOLIN-D 3:20:31	4:14:21 24 HR. BROTH CULTURE PNEUMOCOCCI	RESULT DAYS
281 C*	0	0.2 cc	D 2
20 C	0	0.2	D 1
1	Exp. II	0.2	D 7
11	Exp. I	0.2	S
17	Exp. II	0.2	S
24	4 cc Int	0.2	S
25	3 cc Subc	0.2	D 4
27	3 cc Int	0.2	D 8
30	2 cc Int	0.2	D 7

*C=control D=died S=survived. Pneumocholin-D=equal parts 20 per cent sodium dehydrocholate in aqueous solution and 24 hr pneumococcus broth culture. Int=intravenously Subc=subcutaneously.

to call this antigen "Pneumocholin-D" as cholic acid is characteristic of all the bile salts. "D" represents the dehydrocholate treated pneumococci "T" the taurocholate product, "G" the glycocholate pneumococcus lysate, etc. This experiment indicates that Pneumocholin-D increases the rabbit's resistance. Only one dose of the Pneumocholin-D was given. It remains to be seen whether repeated doses will further increase the resistance.

EXPERIMENT IV

Noneffectiveness of a Beisfeld Filtrate—Pneumococci were grown on blood agar. A heavy suspension of these organisms was made with isotonic salt solution. Then equal parts of the suspension and 20 per cent sodium dehydrocholate

in aqueous solution were mixed and left to stand for three days at room temperature. Lysis was only partial. The mixture was sterile when cultured on blood agar. The mixture was then filtered through a Berkefeld-N filter. The filtrate was divided into two fractions one of which was heated to 60°C for one hour. The two fractions were then injected subcutaneously in rabbits. The unheated fraction was injected in Rabbits 21C, 22, 23, 26, 28, and 29. The heated fraction was injected in Rabbits 274C, 275, 276, 277, 278 and 279. Neither fraction produced any evidence of immunity when the rabbits were given fatal doses of pneumococci.

EXPERIMENT V

Effect of Sodium Taurocholate and Glycocholate on Tissue—The work of this experiment was done with that of a former paper¹ but was unpublished. A 10 per cent concentration of a mixture of sodium taurocholate and sodium glycocholate in 0.85 per cent sodium chloride solution was injected subcutaneously on the abdomen of several rabbits. The injection caused a disagreeable sensation and it was sometimes necessary to make several injections to give the amount of drug desired. These injections caused a local tissue necrosis with eventual sloughing. The inner surfaces of the sloughs when removed were sterile. Local damage to the veins of man by these salts has already been reported.¹ Similar damage and a painful sensation was caused by injecting these salts in the ear veins of rabbits.

DISCUSSION

In a previous paper¹ the theory was advanced that bile salts were used by the body in overcoming pneumococcus pneumonia. This theory was based on the work of Elton⁸ who demonstrated that the icterus index was increased (within the latent zone) in cases of lobar pneumonia. The identification of the bile salts as such in the blood, has not heretofore been possible. There seems to be no reliable chemical test for the bile salts. The Pettenkofer test formerly relied upon is not specific. A new method for the determination of bile salts has recently been advanced by Duco and Panza.¹² The work of Elton on the blood of pneumonia patients should be repeated using a specific test for the bile salts.

In the chemotherapy of such a disease as lobar pneumonia, the question arises as to whether the injected drug reaches the diseased lung. It is believed that it would. Although in a consolidated lung there may be blocking of some arterioles or capillaries, such blocking could not be extensive. Closing of the vessels would result in infarction and certainly this does not occur, at least in those cases which recover. Resolution could not take place if the blood supply to a consolidated area of lung were closed off. Consolidation is produced by exudation into the bronchial system and alveoli and it is probable that the blood supply is never seriously impaired by the consolidation alone. The lung is an extremely vascular organ having a double blood supply.

The nature of the phenomenon of the solution of pneumococci by bile salts is not definitely determined.

The theories to be considered are (1) chemical reaction, (2) catalysis of normal autolysis, and (3) low surface tension.

A direct chemical reaction between the bile salts and some substance or substances in the pneumococcus is the most probable explanation.

Goebel and Avery¹⁷ in 1929 showed that the solution of pneumococci by bile salts is not always accompanied by the same proteolysis that takes place in normal autolysis and that the bile salts act independently of autolytic enzyme. Bile salt solution of pneumococci occurs most rapidly at 37 C. and is retarded both by lower and higher temperatures in the same manner as autolysis.

Low surface tension is not responsible for the lysis of pneumococci as shown by workers already mentioned.⁷

While the explanation of this phenomenon, the solubility of pneumococci in bile salts is not at present definitely known it should not deter the performance of therapeutic investigations with the bile salts.

There seems to be a certain amount of irregularity in the solubility of different strains of pneumococci. Reimann¹⁸ showed that by the repeated growth of pneumococci in plain broth to which was added increasing amounts of bile the organisms became acclimatized to as high as 75 per cent of ox bile. Reimann demonstrated that these organisms after growth in animals were again bile soluble. Also that insolubility in bile was associated with loss of virulence.¹⁸

The study of the amino cholic acids characteristic of bile and their derivatives is opening up a vast field of biologic relationships which needs extensive study. The work of this paper creates more problems than it solves so that definite conclusions are deferred pending further investigation.

SUMMARY

1. Pneumococci of Type I grown on artificial media were not dissolved by sodium dehydrocholate in this work but were killed or rendered avirulent so that they would not grow on artificial media nor produce infection.

2. Sodium dehydrocholate has an antipneumococcus action in the animal body.

3. A mixture of sodium dehydrocholate and of pneumococci when injected into rabbits produces a degree of immunity to pneumococcus infection.

4. Sodium dehydrocholate does not produce local tissue injury as does sodium taurocholate and glycocholate.

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THE DETOXICATION OF COCAINE, Picrotoxin AND STRYCHNINE BY SODIUM AMYTAL*

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KNOEFEL, Herwick and Loevenhart¹ have shown that sodium amytal reduces the toxicity of cocaine, procaine and butyn. Clinically Zerkas and McCullum² have used the drug for cocaine and strychnine poisoning. The purposes of this investigation are to ascertain definitely in experimental animals: (a) Whether or not sodium amytal detoxifies the convulsant drugs which act on the parts of the central nervous system other than the cerebrum; (b) whether or not sodium amytal affords greater protection against poisoning by intravenous than by oral administration; and (c) the efficiency of sodium amytal in preventing the occurrence of convulsions induced by these drugs. Picrotoxin and strychnine were selected for investigation since the former is known to cause convulsions chiefly by the stimulation of the medulla and the latter by that of the spinal cord. Cocaine which is known to produce convulsions by its action on the cerebrum was also studied under the same conditions for comparison.

Rabbits were used in all experiments. The procedure was the same as employed by Knoefel, Herwick and Loevenhart¹ that is the poison is injected subcutaneously and sodium amytal is given simultaneously by vein or by mouth.

As shown in Tables I, II and III, sodium amytal uniformly detoxifies each of the three poisons. Our figures for cocaine with sodium amytal given by vein completely agree with those reported by Knoefel, Herwick and Loevenhart¹. With sodium amytal given orally the MLD of cocaine is 175 mg. per kg. as against 400 mg. per kg. of cocaine when sodium amytal was given by vein. For picrotoxin alone the MLD is 25 mg. per kg. as shown in Table II. With sodium amytal (intravenously) the MLD of picrotoxin is 8 mg. per kg. and with sodium amytal (orally) the MLD is 6 mg. per kg. As shown in Table III the MLD of strychnine alone is 0.6 mg. per kg. With sodium amytal by vein the MLD of strychnine is 4 mg. per kg. and with sodium amytal by mouth the MLD is 2.4 mg. per kg. The detoxifying value of sodium amytal is greater when it is administered intravenously than orally. The difference between the two routes of administration is striking with cocaine and strychnine, but relatively less so with picrotoxin.

There is an interesting relationship in the subcutaneous and intramuscular injection of sodium amytal. With sodium amytal injected subcutaneously (60 mg. per kg.) and intramuscularly (150 mg. per kg.), the MLD of cocaine is the same as with cocaine alone. With picrotoxin and strychnine the prophylactic action of sodium amytal (subcutaneously and intramuscularly) is even greater than by vein or mouth. The MLD of picrotoxin is 15 mg. per kg. with sodium amytal subcutaneously and 10 mg. per kg. with sodium amytal intramuscularly.

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TABLE I
DETOXIFICATION OF COCAINE HYDROCHLORIDE BY SODIUM AMYTAL

WITHOUT SODIUM AMYTAL					WITH SODIUM AMYTAL 10%, 50 MG PER KG, INJECTED INTRAVENOUSLY					WITH SODIUM AMYTAL, 10%, 150 MG PER KG, ADMINISTERED ORALLY				
NO OF ANIMALS	DOSE OF COCAINE IV MG PER KG	NO DIED	NO SURVIVED	ML D IN MG PER KG	NO OF VIALS	DOSE OF COCAINE IN MG PER KG	NO DIED	NO SURVIVED	ML D OF COCAINE IN MG PER KG	NO OF VIALS	DOSE OF COCAINE MILLIV MG PER KG	NO DIED	NO SURVIVED	ML D OF COCAINE
5	25	0	5		5	200	0	5		1	100	0	1	
5	50	0	5		5	250	0	5		1	125	0	1	
5	75	1	4		5	300	1	4		5	150	2	3	
5	100	4	1	100	5	350	1	4		3	175	3	0	175
						400	4	1	400					

TABLE III
DETOXIFICATION OF STRYCHNINE SULPHATE BY SODIUM AMYTAL

WITHOUT SODIUM AMYTAL					WITH SODIUM AMYTAL 10% 50 MG PER KG, INJECTED INTRAVENOUSLY					WITH SODIUM AMYTAL, 10% 150 MG PER KG, ADMINISTERED ORALLY				
NO OF ANIMALS	DOSE OF STRYCH NINE IN MG PER KG	NO DIED	NO SURVIVED	M L D IN VG PER KG	NO OF ANIMALS	DOSE OF STRYCH NINE IN MG PER KG	NO DIED	NO SURVIVED	M L D OF STRYCH NINE IN MG PER KG	NO OF ANIMALS	DOSE OF STRYCH NINE IN MG PER KG	NO DIED	NO SURVIVED	M L D OF STRYCH NINE IN MG PER KG
3	0.3	0	3		2	1	0	2		1	0.6	0	1	
6	0.4	1	5		3	1.5	0	3		1	0.7	0	1	
5	0.5	0	5		3	2	0	3		1	0.8	0	1	
6	0.6	4	2	0.6	4	2.5	0	4		1	0.9	0	1	
3	0.7	3	0		5	3	0	5		1	1.0	0	1	
					5	4	3	2	4	1	1.1	0	1	
					5	5	4	1		1	1.2	0	1	
										4	1.8	1	3	
											2.4	3	2	2.4
											3.0	3	0	

With sodium amytal subcutaneously the MLD of strychnine is 7 mg per kg and intramuscularly the MLD is 5.4 mg per kg. Thus the subcutaneous and intramuscular injection of sodium amytal did not detoxify cocaine. With picrotoxin and strychnine the subcutaneous and intramuscular injection of sodium amytal produced greater protection than by vein or mouth. This discrepancy by the various routes of administration of sodium amytal is not well understood. It may be partially explained by the relative difference in the rate of absorption of these poisons. The development of toxic symptoms of cocaine is considerably more rapid than with picrotoxin or strychnine. Thus it seems that the intravenous and oral administrations of sodium amytal offers a more consistent prophylactic action for all three poisons.

The use of sodium amytal as an anticonvulsant as well as a detoxifying agent was also studied. The minimal convulsant dose (MCD) of each of the three poisons was determined. The average time of onset of convulsions was also observed for the poisons alone and with sodium amytal. No less than five rabbits were used for each dose.

As shown in Table IV and V the average minimal convulsant dose (MCD) of cocaine alone is 50 mg per kg and the average time of onset of convulsions is three minutes. With sodium amytal injected intravenously the average minimal convulsant dose of cocaine is 100 mg per kg. The average time of onset of convulsions with sodium amytal (intravenously) is 150 minutes. With sodium amytal administered orally the MCD is 60 mg per kg and average time of onset of convulsions is fifteen minutes. This shows that sodium amytal injected intravenously is considerably more efficient as an anticonvulsant than when given orally.

The average minimal convulsant dose of picrotoxin is 2 mg per kg with the average time of onset of convulsions as twenty minutes. With sodium amytal by vein the MCD of picrotoxin is 5 mg per kg and the average time of onset of convulsions is delayed to fifty-four minutes. Orally sodium amytal prevents convulsions of picrotoxin up to 4 mg per kg or twice that of picrotoxin alone. The average time of onset of convulsions is seventy minutes. Thus both the intravenous and oral administrations of sodium amytal increased the convulsant dose to twice that of picrotoxin alone and more than doubled the time of onset of convulsions.

With strychnine alone the average minimal convulsant dose is 0.4 mg per kg. Sodium amytal by vein increases this dose to 1.5 mg per kg or approximately 4 times that of strychnine alone. The average time of onset of convulsions with strychnine alone is eighteen minutes. The average time of onset of convulsions is prolonged to forty minutes with sodium amytal by vein and to thirty-five minutes with sodium amytal by mouth. The average convulsant dose of strychnine is 0.8 mg per kg with sodium amytal by mouth. Thus the threshold for convulsions is raised by intravenous injection and oral administration of sodium amytal. Furthermore, the determination of the average convulsant dose of these poisons is less severe with sodium amytal than with the poisons alone. The convulsant dose of cocaine with sodium amytal is so mild that they are with difficulty noted as convulsions. The drawing back of the head and slight spasms are the only symptoms with cocaine under sodium amytal.

TABLE V
AVERAGE TIME OF CONVULSANT DETOXIFICATION OF COCAINE,
Picrotoxin AND STRYCHNINE BY SODIUM AMYTAL

DRUG	WITHOUT SODIUM AMYTAL	WITH SODIUM AMYTAL 10%			
	TIME OF CONVULSIONS AFTER INJECTION (MINUTES)	50 MG PEP KG INJECTED INTRAVENOUSLY		150 MG PEP KG ADMINISTERED ORALLY	
		TIME OF CONVULSIONS (MINUTES)	AVERAGE DIFFERENCE (MINUTES)	TIME OF CONVULSIONS (MINUTES)	AVERAGE DIFFERENCE (MINUTES)
Cocaine	3	150	147	10	7
Picrotoxin	20	54	34	70	50
Strychnine	18	40	22	35	17

TABLE VI
SUMMARY OF TABLES I, II, AND III DETOXIFICATION OF COCAINE,
Picrotoxin, AND STRYCHNINE BY SODIUM AMYTAL

DRUG	WITHOUT SODIUM AMYTAL	WITH SODIUM AMYTAL	
	M L D MG PEP KG	50 MG PEP KG INTRAVENOUSLY	150 MG PEP KG ORALLY
		M L D MG PEP KG	M L D MG PEP KG
Cocaine	100	400	100
Picrotoxin	25	8	10
Strychnine	06	4	54

TABLE VII
SUMMARY OF TABLE IV CONVULSANT DETOXIFICATION OF COCAINE,
Picrotoxin, AND STRYCHNINE, BY SODIUM AMYTAL

DRUG	WITHOUT SODIUM AMYTAL	WITH SODIUM AMYTAL	
	M C D MG PEP KG	50 MG PEP KG INTRAVENOUSLY	150 MG PEP KG ORALLY
		M C D MG PEP KG	M C D MG PEP KG
Cocaine	50	100 to 400	60
Picrotoxin	2	5	4
Strychnine	04	15	08

With cocaine alone the convulsions are severe. Picrotoxin and strychnine under sodium amytal show less severe convulsions than without sodium amytal but are much more pronounced than with cocaine.

The rate of absorption of these poisons varies if the time of onset of convulsions is an indication. Cocaine shows convulsions in three minutes, whereas, picrotoxin and strychnine produces convulsions in twenty minutes as shown in Tables IV and V. The intravenous route of administration of sodium amytal is rapid in its effect, thus producing greater detoxifying properties for cocaine. The oral use of sodium amytal is much slower in its effect and offers less protection in acute poisoning with cocaine. Sodium amytal by mouth gives greater convulsant protection for picrotoxin and strychnine than for cocaine because of more equal rate of development of their effects.

It is difficult to intimate on which part of the central nervous system sodium amytal offers most protection. The differences observed with poisonous drugs may be ascribed to the nature of the substances studied. Thus among the local anesthetics which all act on the cerebrum, Knoefel, Herwick and Loevenhart¹ showed that sodium amytal does not exhibit the same degree of protection.

During the progress of this study, Maloney, Fitch and Tatum³ reported the detoxification of sodium amytal by picrotoxin. This is the reverse of our study. In the detoxification of sodium amytal by picrotoxin Maloney, Fitch and Tatum³ found that approximately twice the M L D of sodium amytal could be given with the picrotoxin treatment. Our findings by the reversible treatment, that is, the detoxification of picrotoxin by sodium amytal was approximately three times the M L D of picrotoxin with sodium amytal.

CONCLUSIONS

1 Protection is afforded by sodium amytal against intoxication of certain poisons that act on the central nervous system.

2 The intravenous and oral administrations of sodium amytal in the order named offers the more effective protection for all three poisons.

3 Sodium amytal is an anticonvulsant as well as a detoxifying agent for the three poisons.

We wish to express our gratitude to Dr. K. K. Chen for suggestions and criticisms.

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THE PRESENCE AND SIGNIFICANCE OF ISOHEMAGGLUTININS IN THE BODY OUTSIDE THE BLOOD STREAM*

BY LELAND W. PARR, BEIRUT, REPUBLIC LIBANAISE

IT IS generally recognized that isohemagglutinins are not confined to the blood stream. They have been found in milk and colostrum¹, saline extracts of malignant tumors², tissue juices obtained via cantharides blisters³, urine⁴, cyst fluids⁵, amniotic fluid⁶, pericardial fluid⁷ and cerebrospinal fluid⁸.

Holzer⁹ has recently called attention to the difficulty which is encountered in determining the blood group of a corpse. He suggests that in such cases the pericardial fluid be utilized. Several cubic centimeters of this liquid are always available and it will be found to contain the same isohemagglutinins as occur in the blood stream. It is sometimes desirable to obtain the blood type of a corpse for medicolegal information or to throw light on a fatal transfusion and for such purposes the use of pericardial fluid is suggested.

The presence of antibodies in the milk and colostrum has long been a topic of interest to immunologists who in so far as colostrum is concerned have worked mostly with the bovine species⁹. In these animals the ingestion of colostrum appears to be a more vital matter than in human beings since in ruminants there is less possibility of placental transmission of antibodies than in an animal such as man which has a simpler placental structure. Heim¹ stated that if the infant were not nursed at the breast the isohemagglutinin content of the serum was affected, agglutinative possibilities being gradually suppressed until in eight to fourteen days the isohemagglutinins would be no more active than in the colostrum in which viscid medium clear-cut agglutination of red blood cells seldom occurs. On the other hand, Happ¹ did not find more isohemagglutinin in the blood of nursing infants than in that of those artificially fed. It is now well known that the isohemagglutinin content of the blood of the newborn child undergoes changes such as have been indicated by several writers¹⁰ but these have to do with the maturation of the dynamic specificities peculiar to the child and determined for it by the factors which it has inherited from its father and mother. Only incidentally is the isohemagglutinin content affected by either placental permeability or mother's milk or colostrum. The child may at first, have some isohemagglutinin derived from the mother, this does not persist, but is replaced by that which its own body has produced. It is being suggested at the present time¹¹ that somewhat the same thing may happen for a wide variety of protein specificities.

Isohemagglutinins may occur in the cerebrospinal fluid although as noted by Kolmer⁸ they are "only occasionally" so found. Herman and Halber⁸ have reported 83 cases in which the cerebrospinal fluid was tested for its isohem-

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agglutinin content They did not find this "antibody" present in normal cases but did find it where there had been organic lesions of the central nervous system in which there was enhanced permeability of the meninges, as in epidemic meningitis tumor of the spinal cord, and multiple sclerosis They did not find isohemagglutinins in the cerebrospinal fluid of cases of tubercular meningitis and they noticed that the isohemagglutinin content of the cerebrospinal fluid was lower than that of the blood serum

At present we regard cerebrospinal fluid as a normal secretion of epithelial origin produced from the choroid plexuses within the ventricles out of which it flows through the foramina of Luschka and Magendie into the subarachnoidal spaces In the sinuses however, the cerebrospinal fluid is brought into very close contact with the venous blood, being separated from it in the Pacchionian bodies only by two thin layers, dura and arachnoid Thus while the normal cerebrospinal fluid is secreted from within the central nervous system itself it may under certain conditions partake of the nature of a transudate

If it could be established that normal cerebrospinal fluid does not contain isohemagglutinins but that in certain diseased conditions it does (provided the blood does), we would have a simple test of reliability which might conceivably be of considerable aid to the clinician

It should be pointed out that where the subject in question belongs to the blood group AB (Jansky IV) there are no isohemagglutinins in the blood and there could not be any in the cerebrospinal fluid from a person belonging to such blood group In our population however, rather less than 5 per cent of the people fall in blood group AB so that it is evident that this limitation is not a serious one

While resident in the Near East we were able to test out the isohemagglutinin content of 104 cerebrospinal fluids In 98 of these fluids no isohemagglutinin could be demonstrated In 32 of the cases we were only provided with specimens of cerebrospinal fluid and were unable to determine the type of the blood Of the 72 whose blood was also typed we found 21 to belong to group O (Jansky I), 34 to group A (Jansky II) 7 to group B (Jansky III), and 10 to group AB (Jansky IV) In the Near East somewhat more than 10 per cent of the people belong to group AB which as we have pointed out is more than twice the percentage in America Six of the cerebrospinal fluids possessed isohemagglutinins and they are summarized in Table I

TABLE I

	CEREBROSPINAL		HISTORY
	BLOOD GROUP	FLUID GROUP	
Case 1	A	A	Typhoid with meningism, Wassermann reaction negative
Case 2	O	O	Tubercular meningitis, Wassermann reaction negative
Case 3	A	A	Compression myelitis, Wassermann reaction positive
Case 4	B	O	Diabetes mellitus and myelitis, Wassermann reaction negative
Case 5	—	O	No history
Case 6	O	A	Undiagnosed fatal case, Wassermann reaction positive

The cerebrospinal fluid and the other fluids shortly to be mentioned were all typed by the open slide method using known red blood cells to establish the group. In practically all cases the typing was in triplicate and the results clear-cut. Hemolysis of the cells used was not observed.

In discussing the data of the above tabulation three points should be noted. In the first place one of the patients (Case 2) was a patient suffering from tubercular meningitis. Herman and Halber did not find isohemagglutinins in this disease but their paper does not state how many cases of tubercular meningitis they had. It is conceivable that their case or cases belonged to the blood group AB in which event no isohemagglutinins would be found in the cerebrospinal fluid even if there were permeability. Secondly, it will be noted in another one of our cases (6) that the blood showed a typing of group O and the cerebrospinal fluid group A. This would mean that one isohemagglutinin "a" failed to pass into the cerebrospinal fluid. It might be that in some cases there is a differential permeability. Or possibly the missing isohemagglutininogen was absorbed by the tissues. Selective permeability has been mentioned by Hirsfeld and Zborowski¹² and Kutsehewski and Schwaizman¹² among others have shown that human tissues and organs have the capacity to absorb isohemagglutinins. Thirdly, it will be observed that we have recorded (Case 4) a patient whose cerebrospinal fluid contained the isohemagglutinins "a" and "b" (Type O) whereas only "a" was found in the blood (Type B). This can only be explained on the basis of some error in technique or record and is one of those cases research workers would like to omit from their protocols did their consciences but permit.

The cerebrospinal fluids examined were all from hospital patients in whom there was some reason for an examination of the fluid because of the clinical history and included twelve specimens from insane hospital patients. In these last no isohemagglutinins were found.

We have also examined ten specimens of ascitic fluid, three of hydrocele fluid, two of synovial fluids from inflammatory conditions of the knee joint, and one specimen of hydatid cyst fluid. All of these were typed as of the same group as the blood with the exception of the fluid from the hydatid cyst. In this case there were no isohemagglutinins in the cyst fluid whereas the patient's blood was of group A.

While the isohemagglutinin content of the cerebrospinal fluid is not quantitatively great, that of the other fluids more nearly resembles blood serum in this respect. Thus a hydrocele fluid obtained November 8, 1925 and typed as a strong group B was still strongly reactive and typically group B May 14, 1928, after more than two and a half years of storage.

SUMMARY

The presence of isohemagglutinin is reported for ascitic fluid, hydrocele fluid, and for synovial fluid obtained from knee joints, the seat of some inflammatory process. No isohemagglutinin could be demonstrated in the one specimen of hydatid cyst fluid studied.

Attention is called to the isohemagglutinin content of the cerebrospinal fluid. It was not present in 94 per cent of our 104 specimens. It is suggested that its

presence may have diagnostic value, to check which further investigation of the point is urged

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THE SIGNIFICANCE OF THE PERIODIC HEALTH EXAMINATION AND ITS INFLUENCE UPON THE HEALTH OF A GROUP OF EXAMINEES*†

SUMMARIZING 500 CONSECUTIVE PERIODIC HEALTH EXAMINATIONS IN PRIVATE
PRACTICE

BY FRANCIS ASHLEY FAUGHT, M.D., PHILADELPHIA, PA.

AN IDEAL method of estimating the value of the periodic health examination would be to compare two groups of persons of approximately similar ages and social status. One group to be examined and told of their defects and abnormalities advised of their importance and instructed regarding their correction and then reexamined after a period sufficiently long to permit of the carrying out of the advice given, the other group to be examined and reexamined after a similar interval but the information obtained withheld so that any improvement in health would be largely the result of individual initiative in efforts to combat actual disease or relieve annoying symptoms. Since such an ideal clinical study is impossible we may, as an alternative, examine a group of apparently healthy persons outline plans for the improvement of their habits and environment and the correction of their defects and then after a sufficient interval to permit of their accomplishment reexamine each one and record the improvements in the individual records as shown by the reexamination, compared with the original.

By this method we should be able to show

- 1 The relative frequency, number and character of complaints, defects and abnormalities found
- 2 The extent of cooperation of each individual as shown by a reduction in the number of defects improvement of habits and the effect of these upon the health of the individual, as shown by the second examination
- 3 The confidence of the public in the periodic health examination as a health promoting measure as indicated by the percentage of original examinees returning for reexamination from which may be determined
- 4 The types of abnormalities and defects receiving the greatest attention by the examinees
- 5 The age period during which persons show the keenest interest in preserving and improving their health

Such data was available in the records of five hundred consecutive periodic health examinations performed by me which furnished the basis for this analysis. They comprise five hundred consecutive health examinations performed in private practice prior to February 1, 1930 and compiled as of February 1, 1931.

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This interval of one year was necessary in order to bring out the elements of the study as outlined above

DATA RELATING TO 500 CONSECUTIVE PERIODIC HEALTH EXAMINATIONS

In Table I, of sex distribution it will be noted that a slightly larger number of women were examined than men. This might be interpreted to indicate that women are more interested in maintaining their health than men. In this series the slight preponderance in favor of the female is probably due to the fact that men found it more difficult to keep daytime appointments for examination, or were loath to spare the two hours' time necessary for study.

TABLE I
SEX DISTRIBUTION

Females	284
Males	216
Total	500

In this group the youngest was eighteen and the oldest seventy-four years

TABLE II
AGES BY TEN YEAR PERIODS

AGE	NO	PER CENT
18 to 20 years	12	2.4
21 to 30 years	76	15.2
31 to 40 years	138	27.4
41 to 50 years	143	28.6
51 to 60 years	88	18.6
61 to 70 years	35	7.2
Over 71 years	8	1.6
	500	100.0

Table II gives the ages by ten year periods. Here, it will be noted that the majority, 281 or 56 per cent are in the fourth and fifth decades, i.e., between the ages of thirty-one and fifty. This fact strongly suggests the existence of a belief among the laity that this is the time when chronic degenerative processes are prone to develop, so that between these ages, the thoughtful individual begins to show a more active interest in his present health and its future possibilities.

TABLE III
NUMBER AND PERCENTAGE RETURNING FOR REEXAMINATION

One reexamination	112	22.2
Two reexaminations	45	9.0
Three reexaminations	24	6.0
Four reexaminations	30	4.8

Table II sets forth the number who have returned for reexamination one or more times, and indicates a definite appreciation of the value of this health-promoting service, otherwise over 22 per cent would not have been reexamined. The fact that 10.8 per cent have returned three or more times indicates clearly that it is possible to develop in intelligent persons, what might be called a "health examination habit."

Chart 1 shows the twenty-three principal complaints in the order of their frequency in 431 or 86.2 per cent of this group. The remaining 69 or 13.8 per cent stated definitely in their questionnaires that they did not have any complaints and that the examination was sought merely as a check-up on their present health.

In addition to the many subjective complaints recorded there was frequently discovered evidence of faulty habits and bad environment that adversely

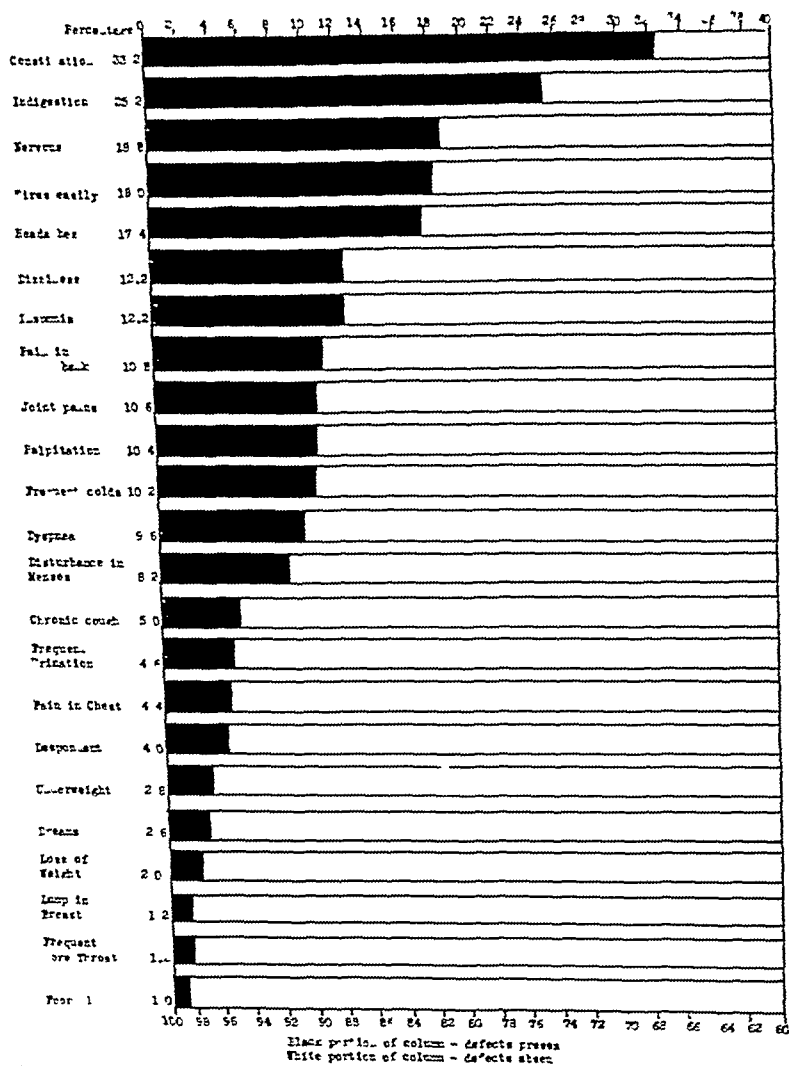


Chart 1—Percentage occurrence of 23 most commonly mentioned symptoms and complaints in 500 examinations

influenced the immediate and future health of the individual. The more important of these in the order of their frequency, and percentage of occurrence are set forth in Table IV.

In studying Chart 2 which sets forth upon a percentage basis twenty-five of the principal defects recorded, it should be remembered that this list is far from complete, since it includes neither all abnormalities discovered nor all that

were reported to the individual at the time of the examination, because it seemed best not to acquaint every individual with the full extent of his defects and abnormalities, as in many instances the report would have been so formidable that it might have discouraged the examinee from doing anything, and therefore, would have defeated the purpose of the examination

SUMMARY 500 HEALTH EXAMINATIONS

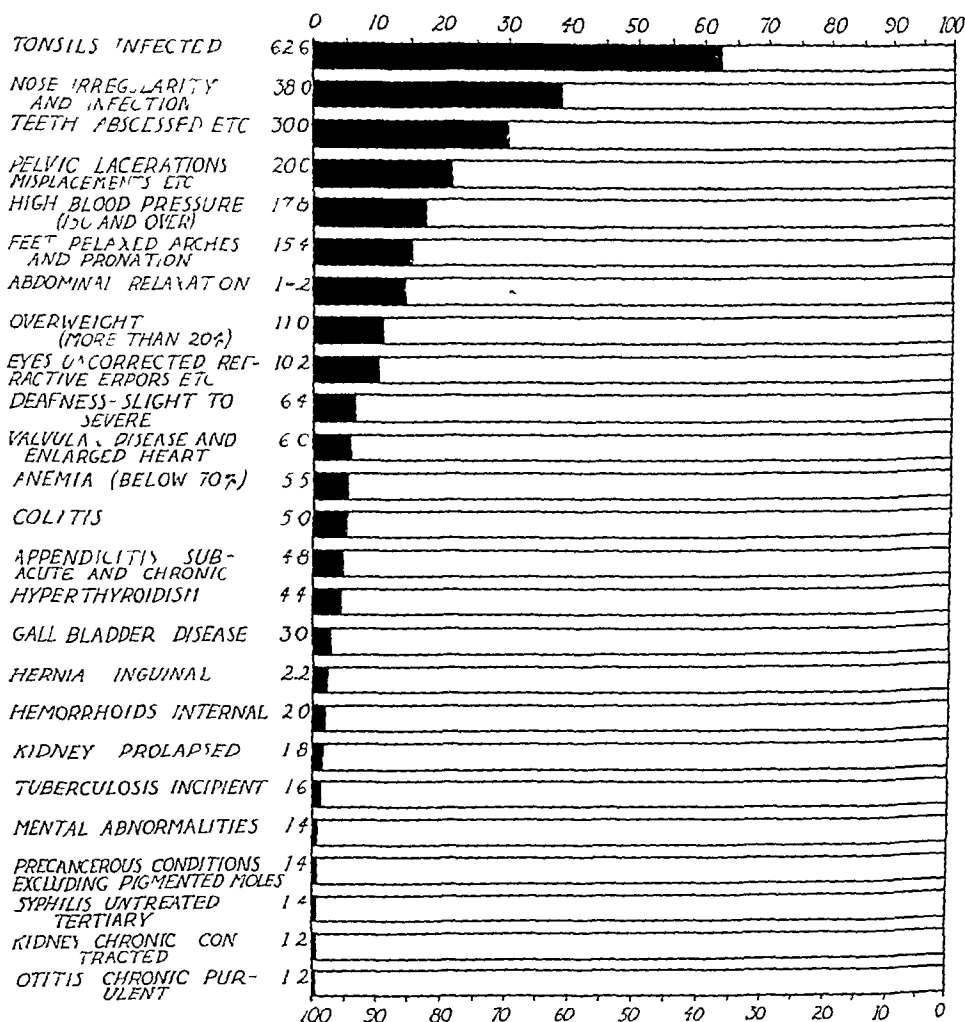


Chart 2—Percentage occurrence of 25 most frequently recorded defects and abnormalities recorded in 500 health examinations

The actual amount of evidence suppressed depended, in a large measure, on the number of defects and abnormalities met in each particular individual, so that even as here recorded it must not be assumed that active intervention was advised in all instances when defects were noted. In this study none were found free of defects, the average number per person was 2.86 and the largest number in any one person was 9.

The criterion applied to determine the inclusion of abnormalities and defects was primarily whether the condition as discovered appeared directly or indirectly to affect the individual's immediate health and comfort or whether if uncorrected or uncorrected they would in a reasonably short time result in disease or be the cause of discomfort or disability. It is important that this fact be borne in mind otherwise there may be some surprise at the relatively small number of defects noted.*

TABLE IV
SOME DETRIMENTAL HABITS REQUIRING MODIFICATION

FINDINGS	NO	PER CENT
1 Insufficient exercise	109	20.1
2 Careless diet habits	63	12.6
3 Insufficient sleep under 6 hours	39	7.8
4 Insufficient fluid intake, less than 50 ounces in 24 hours	28	5.6
5 Excessive use of tobacco		20.0 males only
6 Excessive use of coffee and tea	25	5.0
7 Long working days	19	3.8
8 No recent vacation	18	3.6
9 Excessive hours of work (weekly)	13	2.6

Commenting upon the percentage of chronically infected tonsils found it should be stated that this does not represent the total of infected tonsils originally possessed by this group, because it was ascertained at the time of examination that 75 or 15 per cent had previously submitted to tonsillectomy, so it may be concluded that there were in this series, originally 78.6 per cent of persons with infected tonsils.

THE DATA OF 70 COMPARATIVE EXAMINATIONS

In selecting the records of patients who returned for examination it was found that only seventy of the one hundred and twelve noted in Table III, were available for purposes of comparison, because in the earlier examinations the information recorded was not sufficiently complete to furnish a basis for comparative analysis so that it is upon this comparatively small series that the discussion which follows is based.

The average interval between examinations was 18.8 months, the shortest eight months the longest fifty months.

Chart 3 shows, on a percentage basis the 15 most frequently occurring defects and abnormal physical findings recorded in this group. This chart should be compared with Chart 4, in which appears in the same order the percentage of abnormalities corrected during the interval between the first and second examinations.

In addition, a number of less frequently occurring defects and abnormalities, and a few special examinations were, when advised, corrected treated or investigated in each instance. These included one each of myxedema, internal hemorrhoids perineal abscess gallstones chronic otitis media and ureteral calculus, also one changed an indoor sedentary occupation for one with a maximum

*It should also be noted that many of these health examinations were performed on patients previously under observation and in whom in many instances major corrections had already been made.

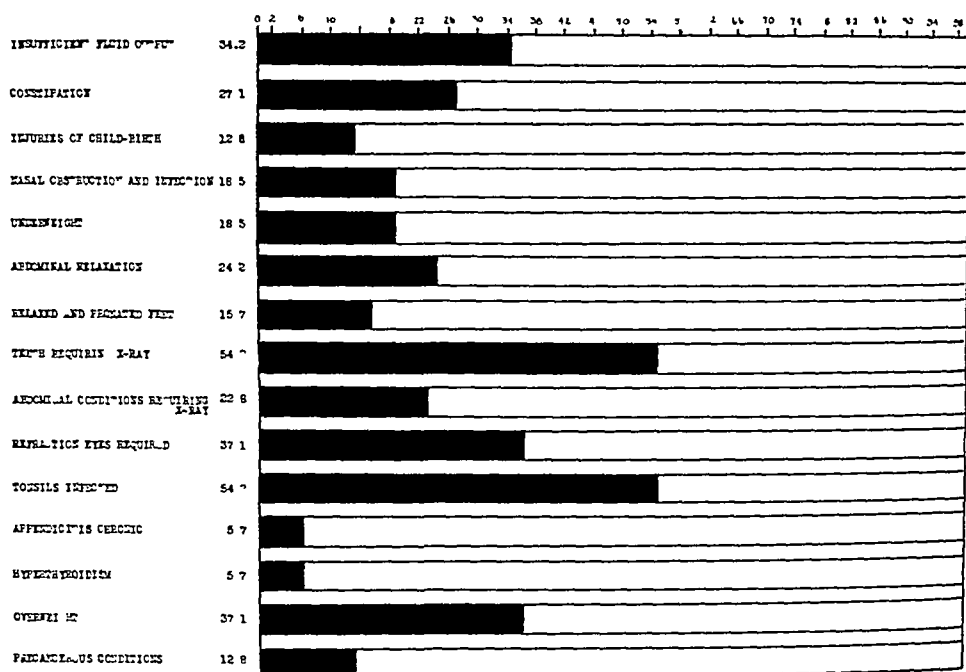


Chart 3—Percentage occurrence of 15 most frequently occurring defects and abnormalities found at first examination

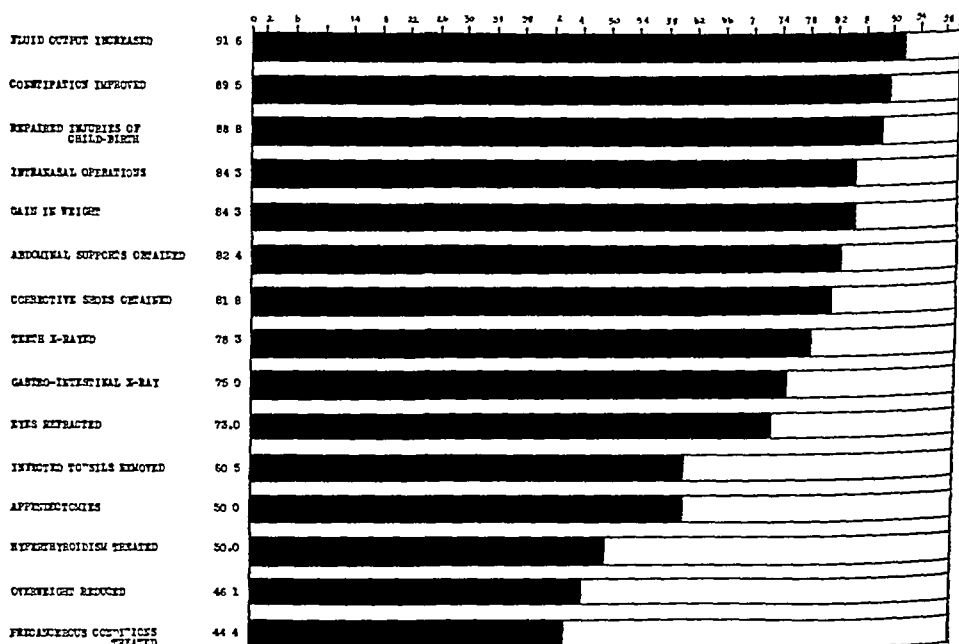


Chart 4—Percentage of defects corrected as noted at time of first reexamination

of fresh air and sunshine, two patients with inguinal hernia adopted the wearing of a truss as an alternative to radical operation one had an x-ray of the chest, and one had an involved joint x-rayed, two were cystoscoped, and there were ten patients treated for anemia (hemoglobin less than 70 per cent) and fourteen had impacted eciumen removed

As a result of their original examination, nine were recommended to have obviously diseased teeth removed while a total of twenty nine were advised to have a partial or complete dental x-ray As a result of the x-ray findings a total of nineteen submitted to single or multiple extractions as a result of which, fourteen patients, finding themselves with insufficient teeth to properly masticate their food, obtained artificial restoration

An unexpected by-product was the apparent effect of generally improved health of the group upon abnormalities in blood pressure There were encountered twenty-five persons whose systolic blood pressure was definitely above the normal average for their age At the reexamination seventeen showed a distinct lowering in high pressure the average reduction being 20 mm Hg, the greatest in any one individual was 55 mm Hg There were also recorded seven cases of hypotension, four of whom showed a definite rise to approximately normal at the time of reexamination Since none of these individuals at the time of their first examination had alarmingly high pressure no specific advice or treatment was directed toward its reduction so that it is rather interesting to note that abnormalities of blood pressure tend without specific treatment, to approximate normal in proportion as the general health of the individual improves, as for example, through the institution of corrective and remediable measures growing out of a periodic health examination

SUMMARY AND CONCLUSIONS

An analysis of five hundred consecutive periodic health examinations is presented, together with 22.2 per cent reexaminations made after a period of one year or longer, 10.8 per cent of these having been reexamined three or more times during a period of about five years

There were slightly more females than males in the series, 56 per cent of the total were between the ages of thirty one and fifty years 13.8 per cent presented for examination with no complaints, but all were found to have one or more defects The average was 2.86 per person, the greatest number of defects in one individual was 9 A tabulation of the more commonly occurring complaints is presented in order of frequency and percentage of occurrence together with the twenty-five most common physical abnormalities found, presented in similar form

A smaller group comprising those who returned for one reexamination is analyzed to show the order of frequency and percentage of occurrence of complaints and abnormalities and in addition the percentage of corrections of abnormalities originally reported as determined at the reexamination, from which may be concluded that

- 1 The periodic health examination definitely appeals to the public, as a means of discovering and arresting those conditions that may later lead to physical infirmity

2 Females and males are about equally interested in the promotion and prolongation of their health

3 The age of greatest interest in matters of health lies between thirty-one and fifty years

4 No adult, upon careful examination, will be found entirely free of physical defects, or without bad habits, and adverse conditions of environment

5 A surprising number of physical defects and abnormalities and detrimental habits may be discovered in apparently healthy persons by means of the periodic health examination

6 Properly presented, there seems to be little difficulty in persuading the individual of average intelligence to proceed with corrective treatment, even when it entails special examinations, radical operations and prolonged treatment

7 There is a distinct tendency toward improvement in hypertension and hypotension, following the removal and correction of abnormalities and defects discovered at the time of examination

8 There can be very little doubt that the systematic discovery of defects and abnormalities and corrections can fail to lead to better health, improved efficiency and longer life

5006 SPRUCE STREET

THE ISOLATION OF AN ORGANISM OF THE ABORTUS-MELITENSIS GROUP FROM A BLOOD CLOT THE SERUM OF WHICH FAILED TO GIVE AGGLUTINATION WITH B ABORTUS*†

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FAILURE to obtain an agglutination reaction with B abortus in the sera of patients having undulant fever has been reported by a number of authors. Only two, however, have mentioned the isolation of the organisms from the blood in such cases. Montagnani¹ (1923) in an article on the intradermal reaction referred to early cases of undulant fever in which no agglutinative properties could be demonstrated in the serum although the organisms were isolated from the blood and an intradermal reaction was obtained. Burnet² (1925) stated that in his experience the absence of the agglutination reaction was more frequent in the sera of patients infected with B melitensis than in those infected with B abortus. Tramontano³ reported the results of the study of sixty-five patients having clinical symptoms of undulant fever, the sera from nine of whom failed to give agglutination with B abortus. He mentioned, however, that ten of the sixty-five cases proved not to be undulant fever. Carpenter and Boak⁴ (1930) indicated that frequently no agglutinative properties are present in the serum of patients suffering from the disease. They mentioned that 6 per cent of the cases which they had studied had fallen into this group. In a personal communication, however, Carpenter stated that he had isolated the organism from the blood of only three of these patients. Giordano and Sensenich⁵ in discussing the literature relative to cases of undulant fever where the blood fails to agglutinate the incitant of the infection, make the following statement: "Our cases suggest this possibility but further investigation of the point is necessary."

Since B abortus has so seldom been isolated from the blood of such cases the report of an additional instance may be of interest.

A specimen of clotted blood was submitted to the branch laboratory three weeks after the onset of illness. No agglutination of B abortus was obtained either in this serum or in one examined two weeks later in the central laboratory in Albany. Tests were also performed with B typhosus, B paratyphosus A, and B paratyphosus B without agglutination being obtained. The blood clot was cultured in about 25 c c of liver-infusion broth P_H 6.8, in a bottle with a rubber stopper and incubated at 37° C for one week. At the end of that time, two plates of liver-infusion agar were inoculated with the broth and incubated in a closed jar in which approximately 10 per cent of the air had been replaced by carbon dioxide. After one week's incubation, colonies of organisms belonging to the abortus melitensis group had developed. Suspensions of the growth were

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agglutinated in a 1:2500 dilution of immune serum prepared with *B. abortus*. The organisms were not agglutinated in the patient's serum.

The history which accompanied the specimen gave the following data. The patient, a housewife of forty-five years, became ill on April 1, 1930, with pain in the chest, headache, vomiting, shortness of breath, and fever varying from 100 to 103°. She was delirious at times. There was no history of diarrhea, constipation, or arthritis. Remissions of temperature occurred every day at about noon, lasting for approximately four hours. The pulse varied from 88 to 120, respiration from 24 to 40. The spleen was not palpable and the lymph glands were not enlarged. In April, 1928 (two years before), the patient had been ill for eighteen weeks, the symptoms noted at that time corresponding exactly with those observed in 1930.

There was no history of contact with goats, cows, or swine, but the patient had drunk raw milk obtained from a herd of cows in which abortions had occurred.

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NORMAL RELATIONSHIPS OF BLOOD AND URINE PHOSPHORUS*

By BURNHAM S. WALKER, PH D., BOSTON, MASS

THE relationships of excretion rates of certain urinary constituents to the amounts simultaneously found in the blood have been extensively studied. This is particularly true of urea for which substance several investigators have proposed mathematical formulations representing the average excretion rates for definite blood levels (Ambard, 1920; Addis and Watanabe 1917, McLean, 1915, Austin Stillman and Van Slyke 1924, Rabinowitch 1925, Adolph 1925, Walker and Rowe, 1927). The relationships observed by these different investigators, while not identical, are in no case widely divergent. Such differences as occur are to be attributed rather to varying approach to the mathematical analysis of the data obtained than to fundamental differences in the observations themselves. It is possible by taking into consideration variations in the experimental procedure to harmonize the results obtained in the case of urea.

This does not seem to be the case as far as the other urinary constituents are concerned, except perhaps in the case of creatinine, where we are dealing with what is for all practical purposes a constant blood level and a constant excretion rate. Surely in the case of phosphorus the problem is as yet by no means clear. This paper is a report of an attempt to observe the normal variations in the excretion of phosphorus and to compare them with the varying content of the blood in the various groups of phosphorus compounds. The general experimental procedure is based on that used in a previous study of urea excretion (Walker and Rowe, 1927) which in turn was based upon the methods of McLean (1915).

Subjects—The observations were made upon sixty students in the first year of their medical course. All were in good health, and had shown satisfactory kidney function with the phenolsulphonephthalein test and by analysis of the blood for the usual nitrogenous constituents. Five of the group were women, no differences in phosphorus excretion attributable to sex were noted hence they have been included in the group without special designation.

Collection of Samples—With the subject fasting, a one-hour urine collection, accurately timed, was completed before 9:00 A. M. Water was allowed as desired by the subject, since this was a study of variations, no attempt was made to standardize water intake or activity. Subjects were permitted to occupy themselves as they wished during the hour of collection. Blood was taken at approximately the middle of the hour period, placed in a bottle containing dry lithium oxalate (one mg. per c.c. blood) and the analysis started at once.

Analytical Methods—In the blood, determinations were made on each sample for inorganic phosphate phosphorus, acid soluble phosphorus, and total phosphorus, using the methods of Fiske and Subbarow (1925) with such slight additions and modifications as described by Walker and Huntsinger (1930). From these analytical results, values were obtained for inorganic phosphate phosphorus,

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acid-soluble organic phosphorus, total phosphorus and lipid phosphorus. The last was obtained by subtraction, rather than by direct analysis (Kav and Brom, 1927, Walker and Huntsinger 1930)

In the urine aside from measurement of the volume of the hour collection, determinations were made of inorganic phosphate phosphorus by the method of Fiske and Subbarow (1925) and of total phosphorus. This latter analysis was carried out as follows: a measured volume of urine (the same volume as taken for inorganic phosphate determination, usually one c.c., occasionally up to five c.c.) was put into a 200×25 mm. pyrex test tube and five c.c. of 10 normal sulphuric acid added. The water was driven off by heating in a beaker of boiling water until charring took place. The remaining acid solution was heated over a micro-burner with the addition of strong nitric acid drop by drop until the solution was clear and colorless, and for about thirty seconds longer. It was then cooled and transferred to a 100 c.c. volumetric flask with about 60 c.c. of water.

To the contents of the flask was added 10 c.c. Molybdate III (Fiske and Subbarow), four c.c. of the usual reducing agent (1-2-4 amino-naphthol sulphonic acid) and water to 100 c.c. volume. After mixing and allowing to stand ten minutes, a reading was made in the Duboseq colorimeter against the usual standard (0.4 mg. P). It will be noted that this procedure is the same as that for total phosphorus in whole blood. It requires less time for the digestion on account of the smaller amount of organic matter present.

The difference, if any, between the inorganic and total phosphorus represents the organic phosphorus in the urine. This method of determination of organic phosphorus by difference is of course subject to the added errors of the two component analyses. A preferable method for larger amounts of urine would be that described by Youngburg and Pucher (1924), in which the inorganic phosphate is first removed by precipitation with magnesia mixture and the filtrate digested. This method requires larger samples than are often obtained in hour specimens.

Inorganic Phosphate in the Urine—The inorganic phosphate varied between a minimum of 0.0256 mg. (as P) per c.c. urine and a maximum of 2.10 mg. per c.c., with a mean of 0.603 mg. per c.c. The median value is slightly lower, 0.522 mg. per c.c.

The maximum of 2.10 mg. per c.c. of urine does not in any way represent a "maximum concentration" in the sense used by Ambard, meaning the limit to which the kidney is able to concentrate a given substance. Wigglesworth and Woodrow (1924), by taking sodium phosphate by mouth reached urine concentrations of 3.7 mg. per c.c., and did not feel that they had reached a limit to the power of the kidney to concentrate phosphate.

Organic Phosphorus in the Urine—The occurrence of organic compounds of phosphorus in the urine is still, despite a massive number of titles on the subject, frequently denied or neglected in books or papers dealing with urinary phosphorus. The weight of the evidence appears to indicate the presence of an appreciable amount of phosphorus in an unoxidized form. The literature up to 1914 is summarized by Forbes and Keith (1914) who state that the organic phosphorus fraction is too large a factor to ignore in any quantitative work.

Youngburg and Pucher (1924) have made an extensive study of the excretion of organic phosphorus using their direct method of analysis. They find an average elimination of 0.131 mg (as P) per kilo per twenty-four hours.

Brain, Kay and Marshall (1928) found organic phosphorus in the urines of 17 normal individuals in concentrations ranging from 0 to 0.028 mg per c.c. using Briggs' method.

The possible pathologic significance of increased organic phosphorus in the urine has been considered occasionally since the time of Zuelzer (1881) and of Lepine, Evmonnet and Aubert (1884). Summers (1904-05) studied the excretion of organic phosphorus in the urine in nine different diseases, finding it significantly increased in lymphatic leucemia and in nervous diseases.

In our series of normal individuals organic phosphorus determinations in the urine were made in 59 cases. In 15 of these no organic phosphorus was detectable. In the remaining 44 cases the organic phosphorus varied to a maximum of 0.195 mg per c.c. The mean value (including all cases) was 0.018 mg per c.c.

The hourly excretion of organic phosphorus varied up to 4.2 mg, with a mean value of 0.729 mg. Assuming a constancy of excretion throughout the twenty-four hours (which is not only unlikely, but distinctly opposed to the finding of an irregular excretion rate for organic phosphorus (Youngburg and Pucher, 1924), yet convenient for a rough comparison of results) the twenty-four-hour output of organic phosphorus would be up to 100 mg. This is a lower value than that observed by Mathison (1910), and is in fair agreement with the figure obtained by Youngburg and Pucher (1924) and quoted above. No correlation between the amount of organic phosphorus eliminated and the blood levels of any of the groups of phosphorus-containing substances was noted.

Excretion Rates of Inorganic Phosphorus—With hourly urine volumes ranging from six to 350 c.c. the amount of inorganic phosphate (as P) excreted during the hour varied from a maximum of 63.7 mg to a minimum of 4.44 mg. The mean value for the hour's excretion was 21.7 mg, the median 18.6 mg.

The simultaneously observed concentration of inorganic phosphorus in the blood in these same cases varied from 2.6 to 4.7 mg per 100 c.c. whole blood. The mean value was 3.6 mg.

The correlation coefficient between concentration of inorganic phosphate in the blood and the rate of excretion was 0.416 ± 0.071 showing that the effect of varying blood concentration on output is apparently less than is the case with urea, where we have shown that the correlation coefficient is 0.840 ± 0.013 (Walker and Rowe, 1927).

The linearity of regression in the case of phosphorus can be demonstrated by calculation of the correlation ratios

$$\eta_{YX} = 0.434$$

$$\eta_{XY} = 0.398$$

which do not vary from the correlation coefficient by more than its probable error.

By plotting the average values of inorganic phosphate output per hour for each blood phosphate level (using intervals of 0.2 mg), we obtain the points shown in Fig. 1. Assuming a threshold for inorganic phosphate at a blood level

of 2.4 mg per 100 cc (Wigglesworth and Woodrow, 1924) the points are well represented by a line (drawn solid) the equation of which is

$$D = 18.2 (B - 2.4)$$

where D is the output of inorganic phosphate in mg per hour and B is the concentration of inorganic phosphate in the blood in mg per 100 cc

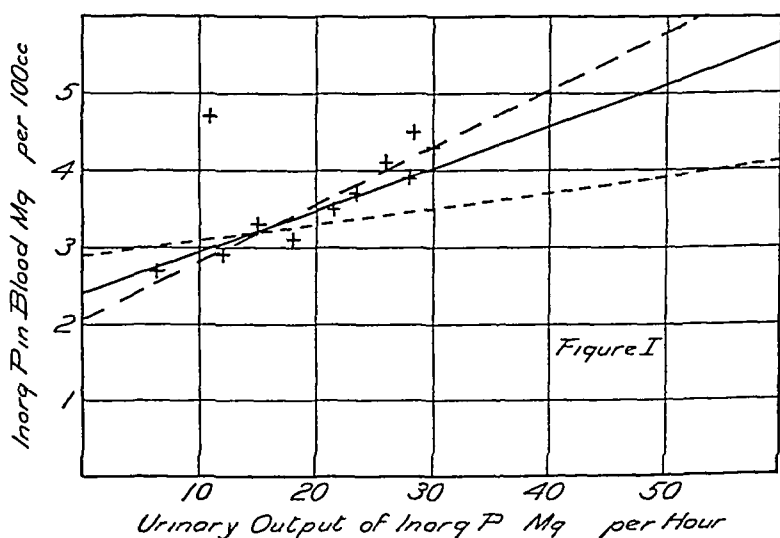
The line drawn in dashes represents the best fitting line regardless of the threshold. This line intercepts the x -axis at about 2.1, fixing the threshold at that point, and giving a somewhat steeper slope. The equation is

$$D = 13.5 (B - 2.1)$$

The dotted line represents the equation

$$D = 50 (B - 2.9)$$

derived by Adolph (1925) from the data of Wigglesworth and Woodrow. In this experiment water was taken in excess. Under such conditions the ratio be

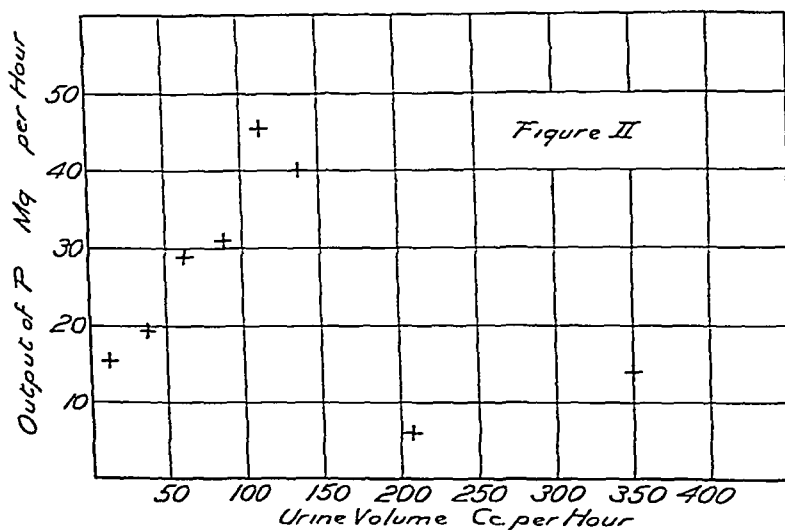


tween the blood concentrations and the elimination rates of urea tends to be stabilized (Addis and Drury, 1923). It seems probable that the difference between the two lines is attributable to the difference in water elimination between our experiments and those of Wigglesworth and Woodrow, and that with increased water output our line would approach the other.

The effect of urine volume on the excretion rate of inorganic phosphate within the limits of variation found in our series seemed at first to be negligible. The correlation coefficient between excretion rate and urine volume was 0.191, a value which can properly be considered as representing no correlation. If the average output of inorganic phosphate be plotted for each urine volume (using intervals of 25 cc) the points shown in Fig. II are obtained. This is suggestive of the existence of an "augmentation limit" for phosphate similar to that defined for urea by Austin, Stillman and Van Slyke (1921). The correlation appears to be built up to about 100 cc and then breaks down completely. Previous

investigators (Wigglesworth and Woodrow, 1924 Harvard and Reay, 1926, Brain Kay and Marshall 1928) have observed the failure of correlation of output with volume all of these investigators have worked with large volumes of water. It seems definitely established that beyond a certain maximum the amount of water eliminated has no effect on the rate of phosphorus excretion. Below 100 c.c. per hour there seems to be a limiting effect of small volume on rate of elimination.

Most modern workers agree to the existence of a threshold for phosphate (see Adolph 1925). Failure of excretion of phosphate below the threshold has not been demonstrated in man. Brull (1927) found that under chloralose anesthesia dogs ceased to eliminate phosphate below a blood level of 4 mg. per 100 c.c. plasma. The threshold suggested by Wigglesworth and Woodrow for man (24 mg. per 100 c.c. blood) coincides well with our observed excretion rates, which allow for a threshold between 20 and 24 mg. per 100 c.c. whole blood.



Other factors than concentration of inorganic phosphate in the blood and rate of water elimination undoubtedly influence the rate of phosphate excretion. The concentration of acid-soluble organic phosphorus compounds in the blood might be expected to have an effect as a result of the action of kidney phosphatase (Brain and Kay, 1929). Since, however, the major portion of this group of compounds is in the corpuscles (Brain, Kay and Marshall 1928) one would hardly expect correlation between whole blood values and urine output. Increase of the ester phosphorus of the plasma by injection of sodium glycerophosphate (Brain and Kay 1929) conditions an increase in the elimination of inorganic phosphate. In our series, we found no correlation between the acid-soluble organic phosphorus of the whole blood and the rate of elimination of inorganic phosphate. The same was true of the lipid phosphorus.

Another variable which we have not considered in this series is the state of the alkaline reserve. With increased necessity for the elimination of acid Haldane Wigglesworth and Woodrow (1924) have shown that there is an increase in the elimination of phosphate.

SUMMARY

In a series of 60 young adults, under normal conditions in all respects except that they were fasting

1 The inorganic phosphate of the urine varied between 0.0256 and 2.10 mg per c.c., with a mean of 0.603 mg (all values given as P)

2 The organic phosphorus of the urine varied between 0 and 0.195 mg per c.c., with a mean of 0.018 mg

3 With urine volumes varying from 6 to 350 c.c. per hour (a) the hourly elimination of inorganic phosphate varied from 4.44 mg to 63.7 mg, mean value 21.7 mg, (b) the hourly elimination of organic phosphate varied from 0 to 4.2 mg, with a mean at 0.729 mg

4 The correlation coefficient between concentration of inorganic phosphate in the blood and the rate of elimination of inorganic phosphate was 0.416 ± 0.071

5 The threshold value for inorganic phosphate lies between 2.4 mg per 100 c.c. whole blood (as suggested by Wigglesworth and Woodrow) and 2.0 mg

6 While there is no correlation between water excretion and phosphorus excretion over the whole series, there seems to be a tendency for simultaneous increase up to an "augmentation limit" at a urine volume of about 100 c.c. per hour

7 No correlation was observed between excretion rates of inorganic or organic phosphorus and the blood levels of the groups of organic phosphorus compounds

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LABORATORY METHODS

THE ASCHHEIM-ZONDEK TEST, MODIFIED, FOR DIAGNOSIS OF EARLY PREGNANCY*

CLINICAL APPLICATION IN 100 CASES

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OUR work in the corroboration of the original Aschheim-Zondek test was begun early in February, 1929, and at that time the original method¹ was closely adhered to. After the performance of possibly a dozen tests, the results of which are not included in this article, we were clearly convinced that for routine work Aschheim's idea of using varied amounts of urine in 5 mice was unnecessary and impractical. The authors, in their original article, frown upon any modification of their technique, but nevertheless we believe that safe results are always obtained with the use of 0.3 c.c. of the patient's urine provided it be secured as a first specimen when the patient arises in the morning. If this specimen is filtered and refrigerated, and kept refrigerated, during the three days' duration of the test, it is always well tolerated by the mice, and will give clear-cut results.

The objects of this investigation can be summarized as follows:

- 1 To verify the high degree of accuracy claimed by Aschheim and Zondek
- 2 To present a series of cases including pregnant females, nonpregnant females, and male controls

- 3 To establish the earliest possible date at which the test is reliable

- 1 *Accuracy*—In our series of 100 cases, 6 were fallacious, giving us an accuracy of 94 per cent. Of the 6 cases in error, the first and second, series Nos. 2 and 87, were questionably positive and repetition was advised. This was possible in the first case, and resulted in a correct reaction. The other (second case) passed from our observation before the test could be repeated. In the third case series No. 80, the urine was apparently toxic, and resulted in the death of 3 of the 5 mice, and the test was positive in only one ovary. In the fourth and fifth cases, series Nos. 38 and 41, the reactions were clear-cut and the discrepancy cannot be explained at the present writing. In the sixth case, series No. 95, a negative gross and histologic result was obtained, the clinically positive diagnosis, however, depending in this case upon the surgeon's opinion of early pregnancy during laparotomy, no follow-up was possible.

It will therefore be seen that the accuracy of the test is probably as high as 98 per cent.

In no case was a positive reaction obtained on male control urine. One case was positive in a nonpregnant female. Five cases were negative in pregnant

*From the Laboratories of Pathology, Medical School, Temple University.
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women In none of these five was the sample the first morning specimen, which fact emphasizes the importance of this detail in technique the hormone being more diluted in the day urine

2 *Synopsis of Series*—The cases of our series were distributed as follows

a Pregnant females, 61 per cent, falling into the groups shown in Table I as to duration of pregnancy

TABLE I

LESS THAN 1 MO	1 MO	2 MO	3 MO	4 MO	5 MO	6 MO	7 MO	8 MO	9 MO
4.8%	11.6%	16.4%	19%	8.2%	6.6%	9.8%	8.2%	8.2%	8.2%

b Nonpregnant females, 27 per cent, showing the following diagnoses functional amenorrhea, 13 cases, amenorrhea following delivery, 5 cases, normal females, 4 cases, psoriasis, 2 cases tumors 2 cases, hydatidiform mole 1 case

c Male controls 5 per cent

d Discarded cases, 7 per cent, due to death of mice or insufficient clinical data

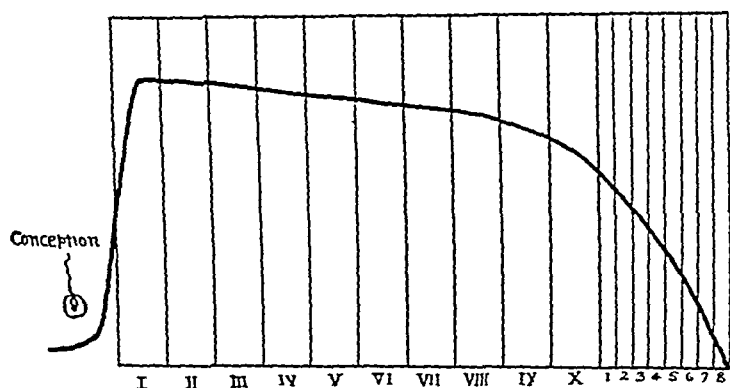


Fig 1—Graphic representation of anterior pituitary hormone discharge during pregnancy and puerperium The ten lunar months are indicated by Roman numerals I to X The eight days of the puerperium are indicated by Arabic numerals 1 to 8 Heavy solid line (—) is curve of anterior pituitary hormone discharge (Redrawn from Aschheim and Zondek.)

3 *The Earliest Possible Date at Which the Test Is Reliable*—Cases were diligently sought in which a suspicion of pregnancy in its earliest days was entertained so that the test could be conducted allowing the lapse of gestation to reveal its accuracy In the following illustration redrawn from Aschheim and Zondek, a graphic representation of the discharge of pituitary hormone is shown as a solid curve It is to be noticed that its maximal height is reached within two weeks following conception, falling off gradually throughout the period of gestation, and very abruptly through a period of ten days after delivery It is to be borne in mind that it is the pituitary hormone hypersecreted during pregnancy, that causes ovarian maturation and ovulation in infantile mice

One case of fourteen days' duration gave us a distinctly positive result, which was proved by date of delivery In the general summary Cases 35, 45, 74, were of less than one month's duration Cases 50, 52, 56, 66 77, 79, 83, were of

TABLE II
DETAILED SYNOPSIS OF CASES

CASE NUMBER	AGE	SEX	COLOR	PARA	DURATION OF ALLEGED PREGNANCY	NIGHT OR DAY SPECIMEN	GROSS REACTION	MICROSCOPIC REACTION	NUMBER OF MICE CROSSLY POS	NUMBER OF BLOOD PUNCTA	ENLARGEMENT OF TUBES AND UTERI	NUMBER OF INFANTIL MICE	CAUSE OF AMENORRHEA
1	22	F	W	1	108 Days	D	+	+	1	5	++	0	Normal Pregnancy
2	30	F	W	2	130 Days	D	-	+	0	0	+	0	Normal Pregnancy
3	31	F	W	4	210 Days	D	+	+	2	10	++	0	Normal Pregnancy
4	21	F	W	2	261 Days	D	+	+	2	9	++	0	Normal Pregnancy
5	21	F	W	3	283 Days	D	+	+	4	15	+++	0	Normal Pregnancy
6	19	F	W	2	99 Days	D	+	+	1	3	++	0	Normal Pregnancy
7	19	F	W	1	146 Days	D	+	+	1	7	++	0	Normal Pregnancy
8	19	F	W	1	164 Days	D	+	+	3	5	++	0	Normal Pregnancy
9	26	F	W	?	?	D	+	+	2	10	+++	0	? ?
10	29	F	W	2	227 Days	D	+	+	3	4	+++	0	Normal Pregnancy
11	34	M	W			D	-	-	0	0	0	5	Normal Male
12	38	F	W	7	166 Days	D	+	+	3	4	++	0	Normal Pregnancy
13	?	F	W	?	?	D	+	+	4	11	+++	0	? ?
14	36	F	W	8	193 Days	D	+	+	2	4	++	0	Normal Pregnancy
15	?	F	W	?	?	D	+	+	5	24	+++	0	Normal Pregnancy
16	24	F	W	1	5 Days After delivery of Mole	N	+	+	2	4	++	0	Hydatidiform Mole
17	19	F	W	1	187 Days	D	+	+	4	20	++	1	Normal Pregnancy
18	26	F	W	2	95 Days	D	+	+	3	12	++	0	Pregnancy—Stillborn
19	15	F	W	1	61 Days	N	0	0	0	0	0	0	•
20	15	F	W	1	76 Days	N	-	-	0	0	0	5	Functional Amenorrhea
21	28	F	W	2	143 Days	D	+	+	5	8	++	0	Normal Pregnancy
22	28	F	W			D	-	-	0	0	0	5	Normal Female
23	25	F	W			D	-	-	0	0	0	5	Normal Female
24	36	M	W			D	-	-	0	0	0	5	Normal Male
25	30	F	W	2	194 Days	D	+	+	2	2	++	0	Normal Pregnancy
26	27	F	W	1	243 Days	D	-	+	0	0	+	0	Normal Pregnancy
27	21	F	W	2	180 Days	D	+	+	3	12	++	0	Normal Pregnancy
28	32	F	W	?	?	D	+	+	1	1	+	0	? ?
29	37	F	W	1	46 Days	N	-	+	0	0	+	3	Pregnancy—Abortion
30	23	F	W	3	166 Days	D	+	+	5	12	+++	0	Normal Pregnancy
31	35	M	W			D	-	-	0	0	0	3	Normal Male
32	26	F	W	4	275 Days	D	+	+	1	1	++	0	Normal Pregnancy
33	36	F	W	3	246 Days	D	+	+	3	4	+++	0	Normal Pregnancy
34	29	F	W	1	153 Days	D	-	+	0	0	0	0	Normal Pregnancy
35	22	F	W	1	21 Days?	N	+	+	2	3	+	0	Pregnancy—Abortion
36	39	F	W			N	-	-	0	0	+	0	Psoriasis
37	28	F	W	3	198 Days	D	+	+	2	2	++	0	Normal Pregnancy
38	20	F	W	1	261 Days	D	-	-	0	0	+++	0	Normal Pregnancy
39	26	F	W	1	214 Days	D	+	+	1	1	++	0	Normal Pregnancy
40	21	F	W	2	225 Days	D	+	+	3	3	+	0	Normal Pregnancy
41	30	F	B	6	226 Days	D	-	-	0	0	+	0	Normal Pregnancy
42	24	F	W	2	274 Days	D	-	+	0	0	+++	0	Normal Pregnancy
43	33	F	W	?	?	D	+	+	1	1	++	0	Normal Pregnancy
44	21	F	W	2	232 Days	D	+	+	4	8	++	0	Normal Pregnancy
45	35	F	B	5	14 Days	D	+	+	2	2	++	0	Normal Pregnancy
46	32	F	W	2	60 Days	N	+	+	5	9	++	0	Pregnancy—Abortion
47	32	F	W	?	120 Days	N	+	+	3	6	++	0	Normal Pregnancy
48	26	F	W	1	60 Days	N	0	0	0	0	0	0	•
49	26	F	W	1	60 Days	N	+	+	3	6	++	0	Normal Pregnancy
50	28	F	W	2	53 Days	N	+	+	2	5	++	0	Pregnancy—Abortion
51	23	F	W	2	113 Days	N	+	+	1	3	++	0	Normal Pregnancy
52	32	F	W	2	49 Days	N	+	+	2	8	+++	0	Normal Pregnancy
53	23	F	W	1	71 Days	N	+	+	1	4	++	0	Normal Pregnancy
54	23	F	W	1	56 Days	N	-	-	0	0	+	0	Functional Amenorrhea
55	28	F	W	7	117 Days	N	+	+	4	11	++	0	Normal Pregnancy

*Mice died during test.

TABLE II (Continued)

CASE NUMBER	AGE	SEX	COLOR	HAIR	DURATION OF ATTENDED PREGNANCY	NIGHT OR DAY SYMPTOM	GROSS REACTION	MICROSCOPIC REACTION	NUMBER OF MICE GROSSLY LOS	NUMBER OF BLOOD FUSCA	INJURY OF TUBES AND UTERI	NUMBER OF INFANTS MICE	CAUSE OF AMENORRHEA
56	24	F	W	1	52 Days	N	+		3	17	++	0	Pregnancy—Abortion
57	20	F	W	1	62 Days	N	+		3	6	+	0	Normal Pregnancy
58	21	F	W	2	92 Days	D	+		2	4	+	0	Normal Pregnancy
59	22	F	W	2	45 Days	N	-		0	0	+	0	Following Delivery
60	41	F	W	1	89 Days	N	-		0	0	+	0	Functional Amenorrhea
61	21	F	W	2	59 Days	N	+		5	14	+	0	Normal Pregnancy
62	21	F	W	3	240 Days	N	+		4	13	+	0	Normal Pregnancy
63	23	F	W	4	103 Days	N	+		1	1	+	0	Normal Pregnancy
64	25	F	W	2	27 Days	N	-		0	0	+	0	Functional Amenorrhea
65	28	F	W	4	63 Days	N	+		4	14	++	0	Normal Pregnancy
66	26	F	W	7	47 Days	N	+		2	9	+	0	Normal Pregnancy
67	22	F	W	4	113 Days	N	+		3	6	++	0	Normal Pregnancy
68	34	F	W	7	49 Days	N	-	-	0	0	0	5	Functional Amenorrhea
69	19	F	W	2	40 Days	N	-	-	0	0	0	5	Functional Amenorrhea
70	28	F	B	6	41 Days	N	-	-	0	0	+	1	Following Delivery
71	39	F	W	1	90 Days	N	-	-	0	0	+	0	Functional Amenorrhea
72	27	F	W	4	74 Days	N	+		4	12	+	0	Normal Pregnancy
73	27	F	W	1	"	N	-	-	0	0	+	1	Following Delivery
74	25	F	W	4	26 Days	N	+		2	12	+	0	Pregnancy—Prem Del
75	24	F	W	4	106 Days	N	+		0	0	+++	0	Tumor
76	35	F	W	4	99 Days	N	+		5	11	+++	0	Normal Pregnancy
77	31	F	W	7	50 Days	N	+		2	4	++	0	Normal Pregnancy
78	30	F	W	6	136 Days	N	+		1	1	+	0	Normal Pregnancy
79	27	F	W	2	58 Days	D	+		2	4	++	0	Normal Pregnancy
80	20	F	W	2	94 Days	D	+		1	1	++	0	Functional Amenorrhea
81	25	F	W	3	96 Days	N	+		2	3	+	0	Normal Pregnancy
82	29	F	B	2	24 Days	D	+		0	0	+	0	Tumor
83	29	F	W	1	42 Days	N	+		4	20	++	0	Normal Pregnancy
84	19	F	W	2	45 Days	D	-		0	0	++	0	Functional Amenorrhea
85	36	F	W	8	77 Days	N	-		0	0	+	0	Following Delivery
86	30	F	W	1	54 Days	N	-		0	0	0	0	Functional Amenorrhea
87	22	F	W	1	65 Days	N	+	+	1	1	0	0	Functional Amenorrhea
88	24	F	W	1	114 Days	N	+		0	0	0	0	
89	24	F	W	1	114 Days	N	+		1	2	++	0	Normal Pregnancy
90	35	F	W			D	-		0	0	0	2	Normal Female
91	33	F	W	1	40 Days	N	-		0	0	0	4	Functional Amenorrhea
92	40	F	W	3	46 Days	N	-		0	0	0	3	Functional Amenorrhea
93	32	F	W	6	56 Days	N	0		0	0	0	0	"
94	27	F	W	6	124 Days	N	-		0	0	0	4	Following Delivery
95	22	F	W	1	86 Days	D	-	-	0	0	+++	0	Pregnancy
96	45	F	W			N	-		0	0	+	4	Normal Female
97	36	F	W			D	-		0	0	0	5	Psoriasis
98	35	M	W			D	-		0	0	0	1	Normal Male
99	36	M	W			D	-		0	0	0	5	Normal Male
100	24	F	W	1	62 Days	N	+		2	5	++	0	Normal Pregnancy

*Mice died during test.

less than two months' duration. Cases 29, 46, 49, 53, 57, 61, 65, 72, 95, 100, were of less than three months' duration, making a total of 33 per cent of the series of less than three months' duration, the period in which clinical diagnosis is impossible or doubtful.

It will be noted that of this series of 20 cases, but one case, Case 95, was incorrect. In this instance the test was negative. The surgeon, on performing an appendectomy, stated that he had observed a pregnant uterus at the time of operation. No follow-up checking was possible.

These findings alone emphasize the value of the test in the early days or weeks of pregnancy, when clinical findings do not merit a diagnosis of the condition. The authors are of the opinion that by using 0.3 c.c. of the first morning specimen of urine positive reactions are obtainable even during the first week of gestation. This would make the test of great value in the recognition of the obscure cases of early ectopic gestation.

TECHNIC OF THE MODIFIED TEST

Apparatus and Materials Required

- 1 One tuberculin syringe, 2 c.c. capacity, kept in a jar of 95 per cent alcohol, for the purpose of sterilization
- 2 Several 3-inch sterile funnels, for filtering urinary specimens
- 3 Package of sterile filter papers to fit funnels
- 4 Test tubes, 150 mm. by 30 mm., fitted with tinfoil-covered corks, sterilized in hot-air oven at 170°C. for one hour



Fig. 2—Photograph (magnification approximately 4X) to show internal genitalia of positive mouse (Serial Case 66) three weeks old, weight 7 gm. Fallopian tubes and ovaries show some enlargement, and are a pale pink color. Note three large corpora hemorrhagica (blood spots) projecting from the surface of the right ovary and one from the surface of the left ovary.

- 5 One battery jar with screen cover, and suitable bedding, etc., to house the 5 mice for each test
- 6 Splinter forceps
- 7 One pair straight manicure scissors
- 8 One hand-magnifier, or low-power dissecting microscope
- 9 One cork board, 6 inches by 12 inches by $\frac{1}{2}$ inch, with pins, for dissection
- 10 Immature or nymph female white mice, less than three weeks old, weighing from 5 to 8 gm., 5 for each test

TECHNIC

The patient is instructed to void directly into the large sterile test tube furnished by the laboratory. At least 25 c.c. of urine should be collected, catheter-

terization is unnecessary. The specimen should be secured on arising in the morning and be immediately sent to the laboratory for refrigeration. At time of the test the urine is filtered in an aseptic manner, using the sterile funnel and sterile filter paper and a sterile test tube for its reception. Refrigeration of the sample must be maintained throughout the duration of the test. The sterile 2 c.c. tuberculin syringe is loaded and each of the 5 mice is injected subcutaneously with 0.3 c.c. of the urine. The injection is made at the root of the tail, on the dorsum of the animal, between the skin and muscle.

Each mouse receives 6 injections of 0.3 c.c. of filtered urine, on three successive days, as follows:

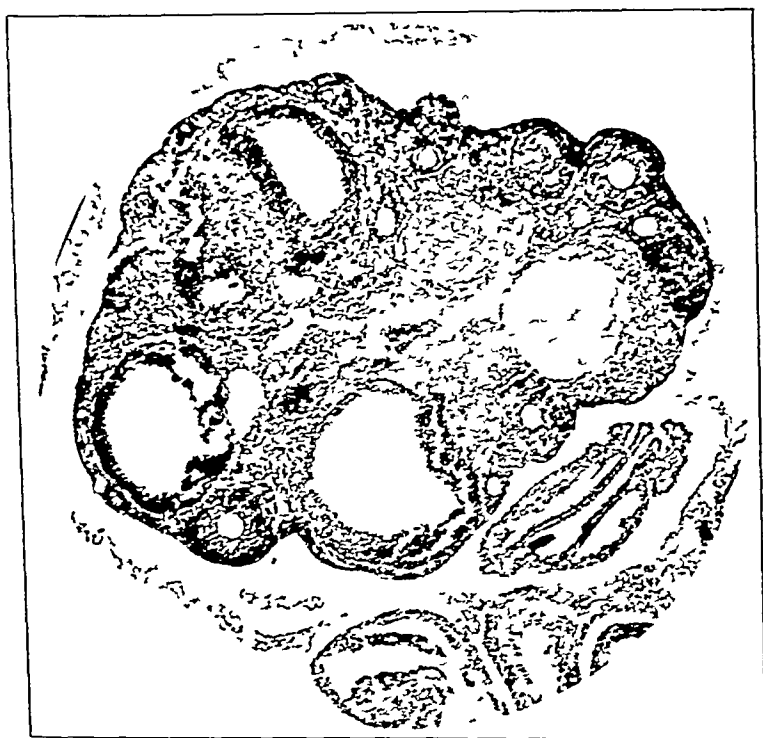


Fig. 3—Photomicrograph (16 mm obj. magnification approximately 100X) of section of ovary from positive mouse (Serial Case 49). Note enlarged mature graafian follicles some showing ova. Also note two corpora lutea, one corpus hemorrhagicum in the upper right hand portion of the section.

Same day urine is received, injections at 1 P.M. and 5 P.M.

Second day, injections at 10 A.M., 1 P.M., and 5 P.M.

Third day, injections at 10 A.M.

On the afternoon of the fifth day, fifty-two hours after the last injection, the mice are killed by passing illuminating gas into battery jars by means of a hose the top of the jar being covered by cardboard.

Dissecting is accomplished by means of the splinter forceps and manicure scissors. Good illumination and the magnifying glass will facilitate the procedure. The incision is made in the median line the intestines being pushed upwards to expose the uterus and tubes. The ovaries will be found in the vicinity of the lower pole of each kidney. They can be drawn down by means

of the splinter forceps, and studied carefully under a dissecting microscope or magnifier, without removal. Positive tests depend upon the finding of one or more black spots from $\frac{1}{2}$ to 1 mm in diameter, projecting as spheric nodules



Fig 4—Photomicrograph (16 mm obj magnification approximately 100X) of section of right ovary mouse No 3 (Serial Case 16). Note numerous mature graafian follicles many with ova. In the upper left hand portion is seen a developing corpus luteum with corpus hemorrhagicum and also two well-marked corpora lutea in lower right portion.

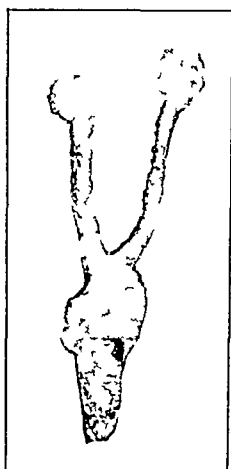


Fig 5—Photograph (magnification approximately 4X) showing internal genitalia of mouse three weeks old weight 7 gm. Note the small size of uterus tubes, and ovaries also absence of follicular development and corpora hemorrhagica (blood spots).

from the ovarian surface. These are the "blut punkten" (corpora hemorrhagica) of Aschheim and Zondek. (See Fig 2). In the absence of these, the test may

still be positive, but requires histologic study of the ovum for revelation of the corpus luteum with or without hemorrhage (See Figs 3 and 4) If the pregnancy test be negative, no gross nor histologic corpora hemorrhagica nor corpora lutea are detectable (See Figs 5 and 6)

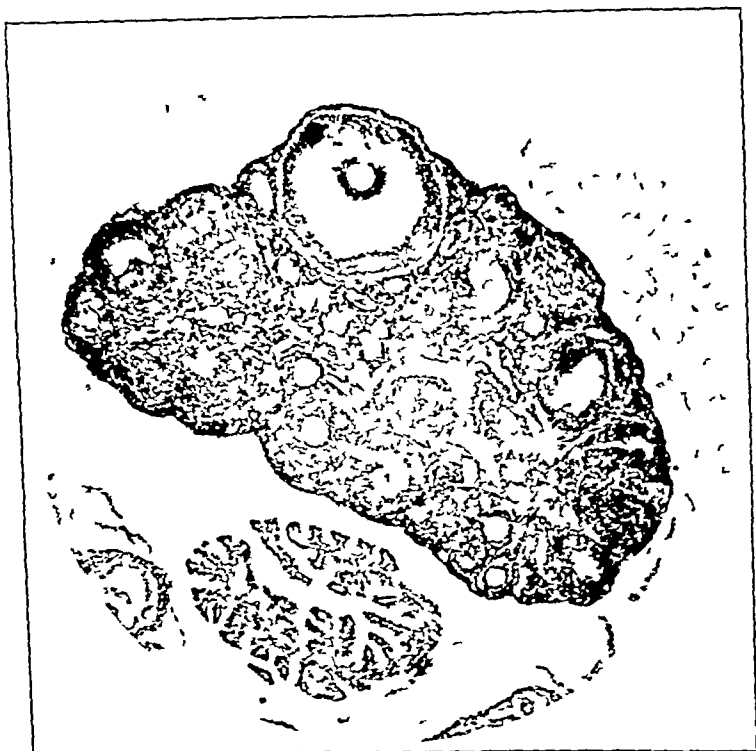


Fig 6—Photomicrograph (16 mm obj magnification approximately 100X) of section from ovary of negative mouse (Serial Case 73). Observe numerous immature small and one large graafian follicle. No corpora lutea nor corpora hemorrhagica are present.

ADVANTAGES OF THE MODIFIED TECHNIC OVER THE ORIGINAL

The use of 0.3 c.c. of the patient's filtered urine given to each of the 5 mice obviates the necessity of labelling or isolating each mouse separately according to dosage. The maximum dose, 0.3 c.c., gives a clearer-cut reaction even when the day urine is used.

CONCLUSIONS

The use of male urine seems to have no effect upon the mice, the uterus, tubes, and ovaries remaining infantile.

In case of hydatidiform mole a positive reaction was obtained.²

Tubal and ovarian maturation in the injected mouse is proportionate, practically in all cases, to the duration of the pregnancy in the woman from whom the urine was obtained.

Unfortunately no cases of pituitary hyperfunction were included in our series.

The possibility of false positives in cases of hyperfunction must be considered but these cases are easily excluded by their symptom complex.

The number of previous pregnancies in each case tested seems to have no influence whatsoever on the reaction

Color, race, and age are without bearing

It is evident in normal female controls, i.e., nonpregnant women with regular menses, that the mice remain infantile after injection, whereas in non-pregnant females suffering with functional amenorrhea, there is a tendency for the uterus and tubes in the mice to be slightly enlarged, and for the hymen to disappear, without however changes in the ovary, the latter being the significant finding in a positive test

The chief disadvantage of the test in the modern clinical laboratory is the difficulty of having on hand immature female mice, less than three weeks old, and weighing not more than 8 grams. A minimum of 15 female breeder mice, and 5 mature males is necessary, but mating is not always controlled, due to seasonal influence

In certain instances the test cannot be performed due to toxicity of the urine causing death of the mice

The number of injections (30), over a period of three days is extremely laborious

The recognition of sex in nymph mice may be difficult to the average pathologist

Dissection requires some skill and anatomic knowledge due to the miniature nature of the organs

Up to date we believe that the Aschheim-Zondek test as herein modified, is the most accurate method for establishing a diagnosis of pregnancy. This method has given the earliest possible diagnosis of pregnancy, and the clinician will see clearly that the test would be of help in the diagnosis of ectopic gestation

The authors wish to express their appreciation to Dr. Harriet L. Hartley, Bureau of Child Hygiene, Philadelphia, and to the various members of the staffs of Temple University and Greentree Hospitals, for their cooperation in furnishing clinical material for this work

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A METHOD FOR THE COLORIMETRIC DETERMINATION OF ARSENIC*

By GUY E. YOUNGBURG, PH.D., AND JASON E. GARBER, BUFFALO, N. Y.

THIS paper presents a rapid but accurate method for the determination of arsenic in biologic materials, organic arsenicals, etc. Although many methods and modifications of them have been proposed in the past, and especially in the last several years, no one has proved to be entirely satisfactory. Evidently improvements are still sought. A comprehensive review of the literature up to 1927 is found in the papers of Minot¹ and Kleinmann and Pangritz.² Since 1927 a number of methods of different types have been proposed.^{3, 4, 5, 6, 7}

We have found that the direct determination of arsenic colorimetrically, after oxidizing its sulphide⁸ is very satisfactory. The organic matter containing arsenic, is oxidized with sulphuric acid, nitric acid and perhydrol and the arsenic is precipitated as sulphide. This is then readily oxidized with sulphuric acid and perhydrol and a blue color is developed by the addition of molybdate and stannous chloride.

Stannous chloride reduction of arsenomolybdic acid gives much more color than other reducing agents which have been used up to the present time. This reducing agent is being used principally for phosphorus determinations,^{9, 10} but is applicable to arsenic.^{11, 12, 13}

Several of the special reagents employed in the following method are those used in this laboratory for phosphorus determinations and the colorimetric procedure is essentially the same. For the sake of convenience we redescribe several of the reagents and a part of the procedure which has been published elsewhere for phosphorus.⁸

METHOD

Special reagents required

1. Ten Normal Sulphuric Acid—450 c.c. of concentrated sulphuric acid¹⁴ is added to 1100 c.c. of water. This solution is titrated and diluted to make 10 normal.

2. Molybdate-Sulphuric Acid Mixture—

Solution A. Mix 50 c.c. of 7.5 per cent sodium molybdate ($\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$) c.p., P-free and 50 c.c. of 10 N sulphuric acid.

Solution B. Mix 50 c.c. of 7.5 per cent sodium molybdate c.p., P-free, 25 c.c. of water and 25 c.c. of 10 N sulphuric acid.

*From the Department of Biological Chemistry, University of Buffalo Medical School. Received for publication July 30, 1931.

¹Although we use a somewhat similar oxidation and sulphide precipitation to that of Fellenberg,⁸ the subsequent process is entirely different. Also the method of Poljakow and Kolokolov¹ bears no similarity to ours except that they finally use a stannous chloride reduction of arsenomolybdate (after Feigl). Their concentrations differ widely from ours since they are not adjusted to give the maximum color possible.

²Sulphide precipitation for the quantitative determination of elements in small amounts is not unusual.^{14, 15, 16}

³While arsenic-free brands of sulphuric acid can be purchased, the c.p. acid used for general laboratory purposes is usually practically arsenic free. Blanks must be run, however.

3 Stannous Chloride Solutions—(Stock and dilute solutions according to Kuttnei and Cohen)

Dissolve 10 grams of stannous chloride, c p (preferably Kahlbaum's) in 25 c c of concentrated hydrochloric acid, c p Store in a brown, glass stoppered bottle

Dilute 0.5 c c of the above stock solution to 100 c c with water This solution is safe to use for a week or until a turbidity forms After that a new dilution should be made

4 Standard Arsenic Solution—Dissolve 0.1533 grams of pure arsenic pentoxide in 50 c c of dilute sodium hydroxide solution (P-free) Neutralize with sulphuric acid and make up to 100 c c with water 1 c c = 1 mg of arsenic

From the above, dilutions are made so that 1 c c = 0.1 mg and 1 c c = 0.01 mg of arsenic Preserve with chloroform These solutions keep for at least six months and perhaps indefinitely

5 Perhydrol—Thirty per cent hydrogen peroxide Merck Blue Label Superoxol is satisfactory since it contains inappreciable amounts of phosphorus or arsenic Keep in the refrigerator after daily use

Procedure

(The following description is for body tissues For other materials analyzed, the approximate amounts to be taken must be estimated)

Fifty grams* of tissue are cut into small pieces, placed in a 500 c c Kjeldahl flask and covered with concentrated nitric acid To it is added 10 c c of concentrated sulphuric acid, several silica† or other pebbles (to prevent bumping) and several drops of caprylic alcohol (to prevent foaming) The flask is then heated, at first gently, then vigorously until fumes from the sulphuric acid appear Oxidation is completed by using more nitric acid and finally perhydrol (by drops, total about 15 c c) The contents of the flask are washed into a 50 c c Erlenmeyer flask, made up to a volume of 20 c c with water and cooled to room temperature Hydrogen sulphide is passed into the cold solution for about five minutes and the flask is then heated to 70-90° while the hydrogen sulphide continues to pass in slowly for an additional five to ten minutes The flask is then stoppered and set aside overnight‡ The supernatant liquid is then decanted, the precipitate is centrifuged and washed by centrifuging with 4 to 6 normal sulphuric or hydrochloric acid 3 or 4 times, except that the last washing is done with water

The precipitate is then transferred to a 150 × 20 mm Pyrex test tube graduated at 10 c c Five-tenths c c of 10 normal sulphuric acid and a silica pebble are added, the tube is placed slanting partly over an electric hot plate or small flame and heated at a boil until the water is evaporated and the acid begins to boil gently The tube is then temporarily removed and after allowing it to cool for about thirty seconds, 1 or 2 drops of perhydrol are added The tube is replaced and the heating continued until oxidation is complete, more perhydrol

*Often less tissue may be taken depending on the amount of arsenic present or the amount of tissue available

†Broken silica-ware can usually be obtained from the organic chemistry laboratory

‡The sulphide is first in a very finely divided state and is slow in settling out If necessary the solution can be centrifuged within an hour after precipitation

being added if necessary. Any possible excess of perhydrol must be removed by continued boiling for five minutes otherwise the color development will be retarded.

The tube is then placed in a rack allowed to cool water is added to the 10 c.c. mark and the contents are mixed. An aliquot (e.g., 5 c.c.) is removed and kept for possible later use.

To the remaining aliquot is added enough 10 normal sulphuric acid to make a total of 0.5 c.c. of that acid and 2 c.c. of molybdate sulphuric acid solution B.

A standard arsenic tube is then prepared by transferring 5 c.c. (0.05 mg As) of standard arsenic solution in a similar tube and adding 2 c.c. of molybdate-sulphuric acid solution A.

To both tubes are then added 1 c.c. of dilute stannous chloride solution and water to the 10 c.c. mark and the contents are mixed without delay. It is best to complete each tube independently.

The color is read in a colorimeter after one minute.

If the color of the unknown greatly exceeds that of the standard dilution may be made, but for most accurate work a smaller aliquot must be taken for the color development.

Calculation

When a 5 c.c. aliquot is taken and the standard is set at 20 mm 20/ reading of the unknown $\times 0.2 =$ mg arsenic in 100 grams of tissue.

DISCUSSION

To oxidize organic matter preliminary to the precipitation of the arsenic as sulphide, we use a modification of the Sanger method, employing sulphuric and nitric acids and perhydrol. While this combustion is time-consuming, it compares favorably with other methods in that regard, and on the whole is the most satisfactory. Fifty grams of beef liver, for example, can be oxidized in one and five-tenths hours.

It is of course impossible to designate the exact procedure for all determinations because of the variety of materials analyzed. Blood is treated like solid tissues. Urine and beverages require concentration to small volume by evaporation as a preliminary process. Organic arsenicals are easily oxidized by sulphuric acid and perhydrol, omitting the nitric acid. Catalytic agents such as ferric chloride, copper sulphate and permanganate cannot be used because they interfere with the sulphide precipitation. Neither is it advisable to use potassium chlorate and hydrochloric acid in the oxidation on account of the volatility of arsenic trichloride.

The conditions for the precipitation of arsenic as sulphide were closely studied. We desire to precipitate in sulphuric acid solution, if possible because the digestion is carried out in this acid. Although hydrochloric acid has customarily been used in qualitative procedure we find that sulphuric acid works equally well. Table I shows how completely the sulphide is precipitated in small quantities and under varying degrees of acidity. It is seen that 0.01 mg of arsenic can be precipitated with a good degree of accuracy from a volume of 20 c.c.

The rate of precipitation varies directly with the concentration of H ions and the temperature¹⁷ However, too much acid, on account of its high gravity, prevents sulphide from being thrown down when centrifuging The optimum acidity lies between 25 and 50 volumes per cent

Some hydrogen sulphide is always reduced in this process, and some free sulphur thus liberated, but this is desirable because it entrains the sulphide and facilitates the handling of it We do not believe that so small a quantity of arsenic as 0.01 mg. could otherwise be recovered

Antimony, which is rarely present, is prevented from precipitating with the arsenic when 40 volumes per cent of acid is used in the precipitation of the sulphide¹⁸ It is later eliminated in the washings along with the phosphate

An intense blue color is developed by arsenic under the conditions described in this method It is 0.4 as intense as the color produced by phosphorus with the same reagents Fig 1 shows that the rate of fading of the blue color is very slow These data were obtained by preparing a standard arsenic tube and then comparing the color with standard arsenic tubes freshly prepared at the times indicated The temperature effect on the color development is so small that it can be neglected

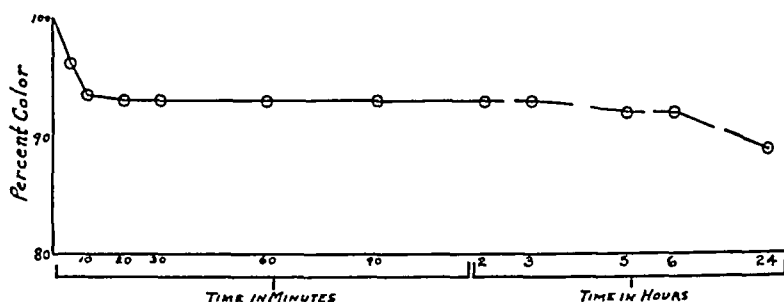


Fig 1

TABLE I

RECOVERY OF ARSENIC FROM PURE AQUEOUS SOLUTIONS OF ARSENIC SALTS WHEN VARYING THE ACIDITY FOR THE SULPHIDE PRECIPITATION

NO	SULPHURIC ACID VOL PER CENT	ARSENIC ADDED MG	ARSENIC RECOVERED MG	RECOVERY PER CENT
1	2.4	1.00	0.997	99.7
2	4.8	1.00	0.997	99.7
3	7.2	1.00	1.003	100.3
4	9.5	1.00	0.985	98.5
5	14.3	1.00	0.995	99.5
6	19.1	1.00	1.040	104.0
7	23.9	1.00	0.985	98.5
8	28.6	1.00	1.000	100.0
9	33.4	1.00	1.000	100.0
10	47.5	1.00	1.020	102.0
11	33.4	0.01	0.013	113.0
12	33.4	0.01	0.010	100.0
13	33.4	0.03	0.028	96.2
14	33.4	0.03	0.031	102.0
15	28.6	0.05	0.049	98.7
16	28.6	0.10	0.096	96.0
17	28.6	0.10	0.097	97.0
18	28.6	0.50	0.499	99.8
19	28.6	1.00	0.995	99.5
20	28.6	1.00	0.997	99.7

TABLE II
RECOVERY OF ARSENIC FROM LIVER

NO	ARSENIC ADDED MG	RECOVERED MG	PERCENT RECOVERY
<i>From 3 Grams of Liver</i>			
1	0.00	0.001	—
2	0.00	0.003	—
3	0.05	0.048	96.0
4	0.05	0.049	98.0
5	0.10	0.098	98.0
6	0.10	0.096	96.0
7	0.50	0.491	98.2
8	0.50	0.488	97.6
9	0.75	0.716	95.5
10	1.00	0.967	96.7
11	1.00	0.943	94.3
12	3.00	2.901	96.7
13	3.00	2.840	94.0
14	5.00	4.800	96.0
15	5.00	4.680	93.6
<i>From 50 Grams of Liver</i>			
16	0.10	0.095	95.0
17	1.00	0.971	97.1
18	1.00	0.954	95.4
19	1.00	0.964	96.4

Table II shows the accuracy of the method in the recovery of arsenic from a tissue. With the smaller amounts of tissue the recovery range is shown to be between 93 and 98 per cent when 5 to 0.05 mg of arsenic is present. With 50 grams of tissue (which may often be taken in practice) the recovery is the same.

Using the standard arsenic solution (not involving the sulphide precipitation) one part of arsenic in 10,000,000 parts can be detected. This is not as sensitive as the Marsh test, but equals that of the Gutzeit method. It is about 250 times as sensitive as the Reinsch test.

SUMMARY

Arsenic is determined by precipitating as sulphide from the oxidized material. The sulphide is then oxidized and determined colorimetrically by the addition of molybdate and stannous chloride.

After the oxidation of organic matter, the method is simple, accurate, and very sensitive.

It is applicable to almost any arsenic determination but was developed primarily for biologic and toxicologic work.

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A NEW PROCEDURE FOR LIGATING THE PYLORUS IN ABSORPTION EXPERIMENTS*

By STEPHEN J MADDOCK BOSTON, MASS

A SURVEY of the methods which have been reported for studying absorption from the stomach reveals that all of them may be included under two general headings (A) acute experiments using general anesthesia or, (B) a series of experiments on animals provided with alimentary fistulas

Employing the first of these general procedures a number of investigators have anesthetized animals opened the abdomen and ligated the pylorus Then they have introduced into the stomach the substance to be studied and after a definite interval have killed the animal and analyzed the material recovered from the stomach at that time By means of such processes much useful information has been collected Thus we shall not attempt to summarize, but merely point out that in connection with such experiments the question always has arisen as to whether or not absorption proceeded at a normal rate while the animals were under anesthesia

The second of the above mentioned general methods has involved the preparation of fistulas giving direct access either to the stomach or duodenum, or both Many of the early investigators used duodenal fistulas and after blocking the distal end of the duodenum with a rubber balloon, caught in a container the material which flowed out from the pylorus Gastric fistulas were used also, and the pylorus blocked by a balloon inserted from above In both instances the difficulty of obtaining quantitative recovery of the unabsorbed material is obvious A number of years ago London (1908 1913) described and used apparently quite successfully, a very ingenious and elaborate double fistula technic for overcoming this last difficulty Quite recently however, a new and hitherto unsuspected element of uncertainty has appeared Gamble and McIver (1925, 1928) have brought to light the deleterious effect of complete loss of gastric or duodenal secretions If there was even a slight but continuous loss of body fluids in the animals with fistulas, it is possible that some abnormality might have been introduced into their response Since the various investigators who have used this device made no specific mention of leakage from fistulas it has not been possible to judge either the extent or the possible significance of this factor

Consideration of the difficulties and uncertainties of the methods previously used led to the design of an experimental procedure which avoided both the complications of ether anesthesia and the effect of fistulous openings into the alimentary tract This method served very satisfactorily in the investigation on carbohydrate absorption which occasioned its development The possible usefulness of it to those who might wish to carry out similar studies upon other substances under conditions so nearly physiologic prompt us to describe the

*From the Biochemical Laboratory of Harvard Medical School
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method in this place. That it is also applicable to the problem of following the early changes in body fluids which follow pyloric obstruction will be evident.

EXPERIMENTAL

The Preliminary Operation—All the experiments in this series were performed upon healthy adult dogs. Under ether anesthesia and with aseptic technic a midline incision 12 to 15 cm long extending downward from the tip of the xiphoid was made. The peritoneal fat was separated and the stomach exposed. The hepatoduodenal ligament was divided sufficiently to allow the pylorus to be brought into the wound. After a little dissection it was possible to pass a flat tape around the pylorus without injury to any of the vessels of the omentum. The ends of the tape were then inserted through a special glass cylinder about 3 to 4 cm in length and of 1.7 cm internal diameter (Fig. 1). These cylinders were open at both ends and the edges were provided with broad

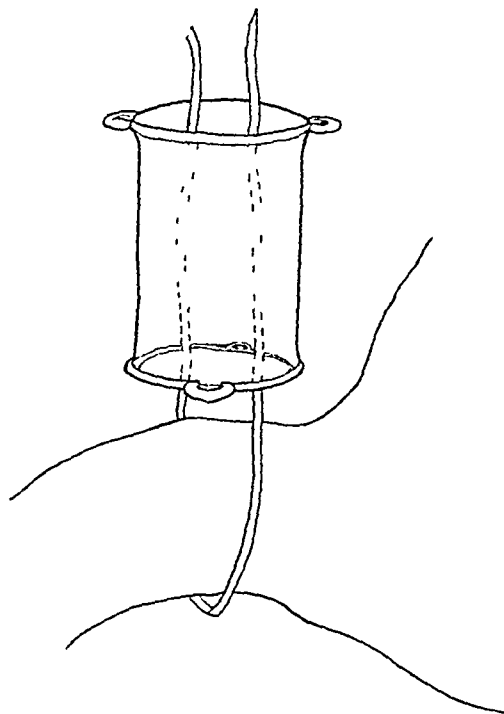


Fig. 1

flanges each of which carried two eyelets in the glass. These eyelets were useful in anchoring the glass cylinder loosely to the pyloric region of the stomach and to the incised edges of the linea alba. Care was taken to insure that the relations of the pylorus were undisturbed and that the glass tubing did not fixate the stomach and thus cause kinking or obstruction. The tape was then anchored loosely to the subcutaneous tissues with its ends protruding from the glass cylinder. The incision was closed with interrupted sutures of silk so that the outer end of the glass tubing was located near the upper angle of the wound. Animals prepared in this way recovered quickly and their appetites and food

consumption promptly became normal. Usually the animals were subjected to absorption experiments within three or four days. However in several instances longer periods elapsed without any noticeable effect upon the results obtained. These animals should not be kept more than six or seven days since the tape tends to disintegrate, and after that period it may break when it is pulled taut.

The Absorption Experiment—After a preliminary fast of eighteen to twenty-four hours a control sample of blood was drawn from the animal. Then by the severance of the stitches holding the skin the ends of the tape and the edge of the glass cylinder were exposed. The snare about the pylorus was slowly and gently drawn tight and fixed by a clamp resting against the edge of the glass tube. A stomach tube was then introduced by mouth into the esophagus and a solution of the substance being studied was allowed to flow into the stomach. The dogs never showed any signs of discomfort and no retching or vomiting occurred for at least two hours. They were allowed to remain on the table or to move about the room. The animals in this series were sacrificed at the end of two hours by the intravenous administration of amytal. In each instance the complete closure of the pylorus was demonstrated at autopsy.

Results—In Tables I and II will be found data which illustrate the applicability of the method for its purpose. Observations upon the blood sugar content of the peripheral veins of dogs before, and just after, the manipulations necessary to close the pyloric snare are contained in Table I. It will be evident that the procedures employed were without hyperglycemic effect. The Folin-Wu method (1919) was employed in making the blood sugar measurements.

TABLE I
EFFECT OF CLOSURE OF PYLORIC SNARE UPON THE BLOOD SUGAR

EXPERIMENT NUMBER	BEFORE	AFTER
	MG PER CENT	MG PER CENT
1	78*	78
2	93	100
3	77	84
4	80	73
5	77	70
6	100	81
Averages	84	81

*All samples drawn from peripheral veins

TABLE II
BLOOD SUGAR CHANGES DURING GLUCOSE TOLERANCE TESTS

TIME	PYLORIC SNARE	
	(A) OPEN	(B) CLOSED
MINUTES	MG PER CENT	MG PER CENT
Control	72	78
25.5 gm Glucose in 200 cc Water Introduced Into Stomach		
15	93	83
30	105	85
60	149	82
90	165	87

Weight of dog 8.5 kilos
 Pyloric snare introduced Nov 11 1928
 Experiment (A) Nov 17 1928
 Experiment (B) Nov 20 1928

Table II records the data of some blood sugar studies made upon a dog after a pyloric snare had been put in place as described above. In experiment (A) the snare had been in position for several days, but had not been closed. In experiment (B) the snare was closed after the control blood sample had been taken but before the sugar was introduced.

In the experiment in which the snare was in place, but open it may be noted that glucose produced a normal rise in the sugar content of the peripheral blood. On the other hand when the experiment was repeated a few days later with the snare closed, the sugar fluctuated only slightly and an increase in excess of the uncertainties of sampling and measurement was not obtained.

SUMMARY

A procedure is described by which the pylorus can be obstructed when desired without the complication of general anesthesia. A snare, consisting of a glass tube and a flat tape encircling the pylorus, is inserted at a preliminary operation. This is allowed to remain in place for several days while the animal recovers. Then by means of this device the pylorus can be closed readily from the exterior just before an experiment is to be undertaken. A series of blood sugar values is reported which show the application of the method to the study of the absorption of glucose from the stomach.

This procedure has been developed during the course of an investigation carried on in collaboration with Professor Harry C. Trimble.

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A SIMPLE METHOD FOR THE ANALYSIS OF PROTEIN IN MILK*

By CHARLES F. CROWLEY, A. M., Ph. C., M. D., LL. D., OMAHA, NEB.

THOSE fat removed from well-equipped laboratories may have occasion to require the protein content (casein, lactoglobulin and lactalbumin) of milk when facilities for the Kjeldahl method are not available. Reliable results may be quickly and easily obtained by the following procedure, using a centrifuge and 15 c c tubes.

Centrifuge 15 c c of milk and remove the cream layer. It may be thrown off with a sudden jerk over the sink or with a pipette the skimmed milk can be drawn from below. Dilute 5 c c of this skimmed milk with 20 c c of water and shake well. Place 5 c c of the dilution in a Purdy 15 c c centrifuge tube add 5 c c of water and 5 c c of Esbach's picric acid solution then shake well.

ESBACH'S SOLUTION

Picric acid	1 gm
Citric acid	2 gm
Water	100 c c

Place tubes in the centrifuge and run centrifuge for just three minutes. The number of cubic centimeters of precipitate in the conical bottom of the tube multiplied by 6 will approximate the amount of protein in the milk in percentage.

The centrifuge used in our experiments has a diameter of 15 inches and a speed of 1,300 revolutions per minute. Milk containing, by the Kjeldahl method 3.5 per cent of protein gave a volume precipitate of 0.6 c c in the conical end of the tube. This multiplied by 6 gives 3.6 per cent of protein.

Whole milk cannot be used in the above method until the fat is removed, as the amount of fat swells the volume of the precipitate and is sometimes so large as compared to the protein, that a floating instead of a sinking coagulum is formed.

COMPARISON TABLE

KJELDAHL METHOD		CENTRIFUGE METHOD	
3.50	Cow's Milk	3.60	
3.43	Cow's Milk	3.30	
3.31	Cow's Milk	3.15	
3.32	Cow's Milk	3.15	
3.50	Cow's Milk	3.30	
2.27	Cow's Milk	2.40	
2.10	Cow's Milk	2.40	
3.06	Goat's Milk	3.00	
3.06	Goat's Milk	3.00	

With a centrifuge having a less diameter and a less number of revolutions per minute a smaller constant or multiplier than 6 would be used as the volume of the precipitate would be larger. The three factors controlling the volume of

*From the Department of Public Affairs, Omaha.
Received for publication December 19, 1929.

the precipitate are diameter of the centrifuge, speed, and time. These can be constant for anyone carrying on the above method.

The merit of this method is in its availability irrespective of diameter, speed, and time, inasmuch as one can determine the volume of the precipitate for a sample of normal milk, with his particular centrifuge, with its particular diameter and speed and specific time of three minutes. Any sample having a less volume will of course be below normal, while greater volumes are above normal. This is true even when the tubes are not very correctly graduated as is sometimes the case.

Should other components of milk be of interest the following details can be followed.

Take the specific gravity with the urinometer spindle. Variation is usually between 1.025 and 1.035. Always make correction for temperature on the basis of one gravity degree (third decimal place) for each seven Fahrenheit degrees above or below 60° F. as the spindles are standardized for 60° F. Add if above, and subtract if below.

Determine the fat content by the Babcock method. Use 5 cc of milk, add 5 cc of sulphuric acid (sp. gr. 1.80), one cc at a time, shake well after each addition. Centrifuge for three minutes, add hot water to force the fat up into the measuring neck of the tube, centrifuge one minute and read the amount of fat.

Total solids can be found by Richmond's formula: $12 \times \text{Fat} + 0.25 \times \text{Gravity (second and third figures)} + 0.14 = \text{Total solids}$. Example: Sp. gr. = 1.032 and Fat = 3.8 per cent. $12 \times 3.8 + 0.25 \times 32 + 0.14 = 12.70$ Total solids.

The total solids can also be determined by weighing a dish with 10 gm of sand and a stirring rod, adding 5 gm of milk, placing in an oven at 100° C and evaporating to constant weight. Increased weight multiplied by 20 gives the percentage of solids.

The ash may be determined by evaporating 5 gm of milk in a small crucible to dryness and then incinerating. The increased weight of the crucible multiplied by 20 will be the percentage of ash, or inorganic solids.

It is quite evident that a sugar determination is not necessary as the sum of the protein, fat, and ash subtracted from the total solids would equal the lactose.

Milk Sugar—Lactose may be determined by taking 5 gm of milk and adding 40 cc of water and 5 cc of glacial acetic acid. Boil, cool, and make volume up to 50 cc. Filter this mixture into a burette and from it run the solution slowly into boiling Purdy's ammoniacal copper solution, until the blue color just disappears. Use 35 cc of this solution.

PURDY'S AMMONIACAL COPPER SOLUTION

Copper sulphate	4.752 gm
Caustic Potash (KOH)	23.500 gm
Ammonium Hydroxide (sp. gr. 0.9)	350.000 cc
Glycerol (C ₃ H ₅ OH) ₃	38.000 cc
Water to	1000.000 cc

Since 35 cc of this solution is reduced by 0.02 gm of glucose or by 0.03 gm of lactose, it is only necessary to divide the number of cubic centimeters required to decolorize the 35 cc of Purdy's solution into 0.03 and multiply by 100 to obtain the percentage of lactose in the dilution and by 10 again, as the 5 gm of milk were diluted to 50 cc. Of course Benedict's solution can be substituted for Purdy's, but remember that the 25 cc of Benedict's solution is reduced by 0.05 gm of glucose and 0.067 gm of lactose.

Where a polariscope is available such as Ultzman's, measure out 25 cc of milk, add 0.5 cc acid mercuric nitrate solution (made by dissolving mercury in twice its weight of nitric acid sp. gr. 1.42 and diluting with an equal volume of water) and 2 cc of water. Shake, let stand five minutes, filter and polarize. Multiply reading by 11. The specific rotating power of glucose and lactose are about the same at 20° C, viz, 52.5.

Inasmuch as the foregoing directions are for rapid work and the results are approximations, it makes little difference whether 5 cc of milk are used where 5 gm are indicated, and vice versa.

A PERMANENT NITROPRUSSID SOLUTION FOR ACETONE TESTS*

By ROBERT M. HILL, PH D, DENVER, COLO

IN URINALYSIS acetone tests which make use of nitroprussid can have no quantitative significance and have rather questionable comparative value unless the nitroprussid solution be of the same strength for each determination. A water solution of nitroprussid decomposes so rapidly in the light as to make the quantitative use of such a solution impossible. Semiquantitative methods based upon the nitroprussid reaction have lacked much in convenience because of the necessity of preparing the reagent each day with quantitative care. Because this procedure was irksome and because of the loss of time involved, a study of the problem of stabilizing the nitroprussid solution was undertaken in this laboratory about two years ago. After many failures one successful method was found and it alone will be discussed.

It was thought that the free acid might be more stable in solution than the salt. Two per cent solutions of sodium nitroprussid were made in sulphuric acid[†] of strengths ranging from 1 to 5 per cent. Samples of these were put in clear glass and in brown glass bottles placed on the laboratory shelf and examined from time to time. Deterioration was measured by the appearance of Prussian blue and by color comparison with a freshly made solution. These solutions have been standing now for eighteen months portions being used from time to time for acetone tests. Only the solution in one per cent sulphuric acid in the clear glass bottle has shown any deterioration. A trace of blue color appeared in the bottom of this bottle during the eleventh month. The nitroprussid in one per cent acid in the dark glass bottle is still as good as when prepared. Ten per cent solutions of sodium nitroprussid in 1, 2, and 5 per cent sulphuric acid are still as good as when made, after six months in brown glass bottles. Since both the brown bottles and the sulphuric acid contribute to the stability of the solution we now use either 2 or 10 per cent sodium nitroprussid in 2 per cent sulphuric acid and keep the solution in a brown glass bottle. This amount of acid is of no consequence in using the qualitative acetone methods, in quantitative methods a corresponding excess of alkali may be used.

Recently it has come to our attention that Cavalli (1897) and Zucarni (1915),[‡] both inorganic chemists have reported that nitroprussid solutions are far more stable when kept in the dark and that small amounts of sulphuric acid added to the solution greatly increase this stability.

For the convenience of physicians, 10 per cent sulphuric acid made up with quantitative accuracy may be obtained from the Denver Fire Clay Company of Denver, Colorado.

*Received for publication July 27, 1931.

[†]Hydrochloric acid decomposes nitroprussid quickly producing hydrocyanic acid. Nitric acid was not used because of its oxidizing action.

[‡]Cited by J. N. Friend, A Textbook of Inorganic Chemistry, Vol. IX, Part II, p. 231.

A SPLIT SECOND TIMER*

B. C. C. GUTHRIE, M. D., PH. D., PITTSBURGH, PA

FOR many laboratory purposes it is desirable to measure time in fractions of a second as with stop watches. Aside from their high first cost, short life in student hands and high cost of and unsatisfactory character of repairs of the usual Swiss works, they are convenient and satisfactory.

To eliminate some of these objections, an inexpensive, simple and accurate timing device has been designed to supplant watches for many such purposes.

It consists of a glass reservoir and graduated glass tube connected by a metal tap mounted on a rotating base, and a suitable quantity of cleaned and sifted sand. (See Fig. 1.) It is at least as accurate as the average stop watch.

To operate, the base (8) is rotated upon the friction support (6) until the reservoir (1) is down and the base is against a stop on the support and is nearly perpendicular. The tap (3) is opened and all sand in the graduated tube and connection is emptied into the reservoir. The tap is then closed and the base rotated to perpendicular when it comes in contact with the support stop and the reservoir is up. The timer is then ready for use, the tap being opened at the start and closed at the end of an observation. The time is read on the scale from the top of the sand column in the tube.

After the best coarseness of sand had been determined an article dealing with the most favorable coarseness of fertilizers for uniform distribution by machines came to notice.¹ Such materials having an average angle of repose of about 34° gave the best results.

The angle was determined by pouring the material into a conical pile upon a horizontal surface and measuring the angle between the surface and cone. This was done with the sand used in the timer and it measured within about a degree of that of the fertilizer.

*Received for publication July 22, 1931.

¹Mehring, A. L. *Industrial and Engineering Chemistry* 3: 34, 1931.

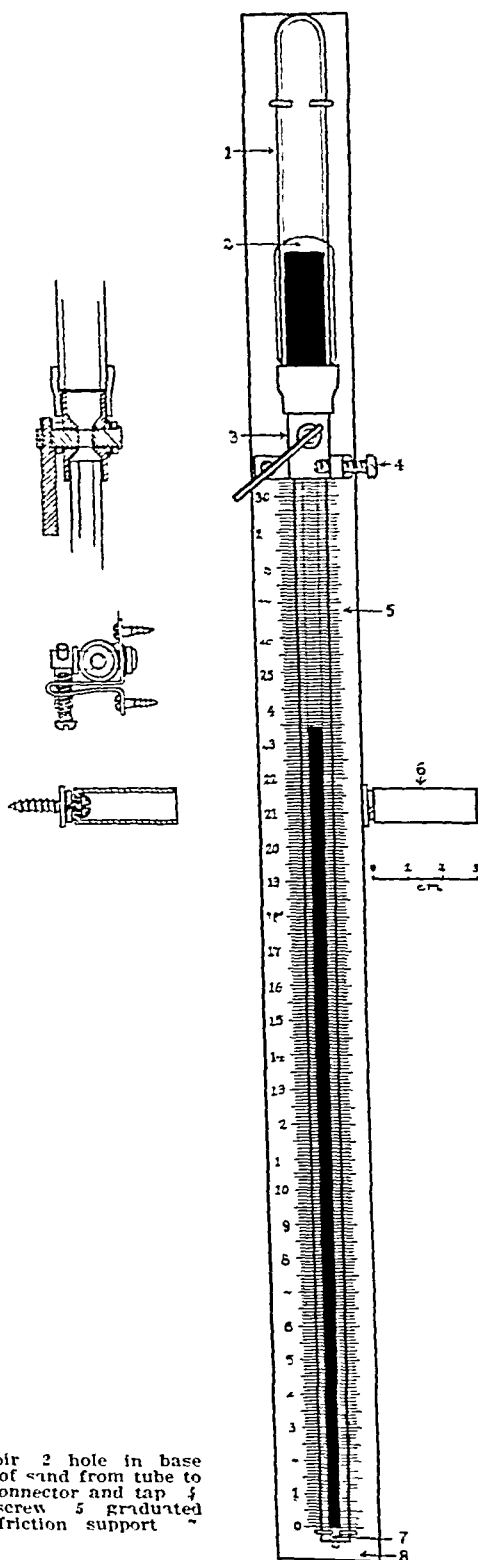


Fig 1—1 Reservoir 2 hole in base for observing return of sand from tube to reservoir 3 metal connector and tap 4 adjusting and stop screw 5 graduated tube 6 rotating friction support 7 plug 8 base

A NEW DEVICE FOR HONING MICROTOME KNIVES ON GLASS*

By N GRAHAM STABLER, PHILADELPHIA, PA

A DEVICE known as the *Schmid hand microtome knife sharpener*[†] has recently been placed on the market. The device makes use of a glass plate for honing, as does the Fanz automatic knife sharpener,¹ but is very simple in design and therefore very much less expensive. Compared to honing on stones, much time is saved, much less skill is required, and there are other advantages, inherent to all glass plate honing methods, which will be mentioned below.

This device consists (see Fig. 1) of a glass plate 14 by 14 inches, 1/2 inch

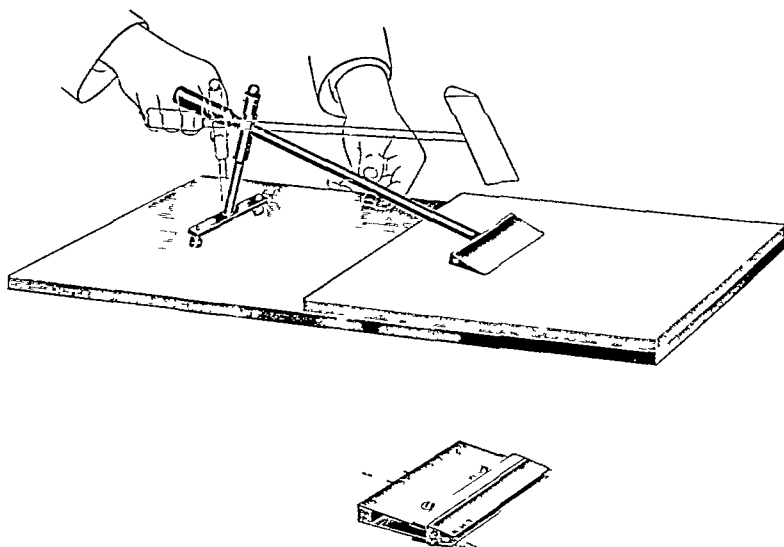


Fig. 1 —The microtome knife sharpener and clamp

thick, ground on one surface, on which the honing is done, a glass base plate 14 by 28 by 1/2 inches, a phosphor-bronze clamp which will hold almost any microtome knife of detachable handle type, a stainless steel knife holder rod which screws into the clamp and has a convenient wooden handle on the other end, a stainless steel upright with two casters and with eight holes, any one of which can be used for the knife holder rod. The latter screws apart at the point where it fits the upright, and will rotate freely in its hole in the upright when in place. The outfit as sold includes in addition a set of abrasives and accessories. The knives furnished from now on by the maker of this device will have a threaded hole for the knife holder rod. With these knives the clamp is not needed.

*From the McManes Laboratory of Pathology of the University of Pennsylvania
Received for publication August 12 1931
†Sold by Arthur H. Thomas Co. Philadelphia

The method of using is very simple, and while it requires reasonable care on the part of the user, it requires practically no skill. An inexperienced girl has made a knife sharp the first time by following the written directions that come with it. For working a knife into shape the first time and for rough honing FFF emery flour is suspended in 5 per cent glycerin and poured on the honing plate and the knife is worked rapidly in circles on it. For finish honing white rouge is used, suspended in 5 per cent glycerin and more care is exercised in honing, the knife being pushed edge first only. Anyone who so desires may use diamantine No. 1 for finer honing. It has recently been found, however, that an absolutely smooth edge is undesirable, at least for paraffin cutting, and it is believed for celloidin also. Experience indicates that there is an optimum degree of roughness of the edge for best cutting, and that white rouge seems to produce somewhere near this optimum degree. A knife honed on white rouge and stropped slightly cuts beautifully. It is the sharp uniform-grade abrasives that make glass plate honing speedier than even well-dressed stone hones.

The device seems to merit wide use because first, its low cost, not a great deal more than a set of hones, puts it within the reach of every laboratory, second, because no user should have trouble developing what skill is necessary, third, because the honing surface will not wear out of shape as do stone hones; fourth, because any grade of abrasives desired may be used. Its only disadvantage, breakability of the glass, is not serious. The thick plate glass does not break easily, and is not very expensive to replace. This disadvantage is inherent in all glass plate honing methods.

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A DEVICE USED WITH MOUNTED INTESTINAL SPECIMENS TO SIMULATE SIGMOIDOSCOPIC VIEWS*

By JOSEPH FELSEN, M.D., NEW YORK, N. Y.

PURPOSE—Didactic. A simple optical arrangement to be used in connection with mounted intestinal specimens to simulate views obtained through a sigmoidoscope or proctoscope.

Principle—A reflected image of a limited area of the specimen is viewed through a cardboard tube.

Apparatus—(1) A well silvered plane surfaced mirror of high optical quality. (2) Gray cardboard tube of approximately the same length and diameter as a sigmoidoscope (12 by $\frac{3}{4}$ inches) or proctoscope (6 by 1 inches).

The mirror should be approximately six inches in its anteroposterior diameter and set at an angle of ten to fifteen degrees to the horizontal plane. The viewing tube, held by wire, cardboard or aluminum combination shield and sup-

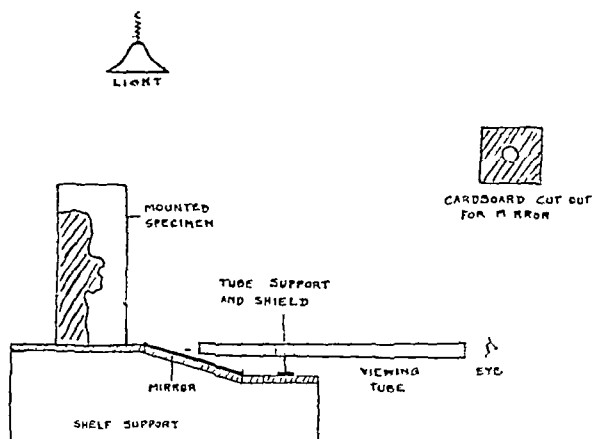


Fig. 1—A pseudosigmoidoscopic device adapting mounted intestinal specimens for clinical study.

port, is placed horizontally in such a position as to afford a view of the pathologic area to be demonstrated. The purpose of the shield, which is approximately the size of an ordinary filing card (3 by 5 inches) and which is slipped over the viewing tube between the eye and mirror, is to shut out confusing images of objects in the vicinity or of contiguous portions of the specimen. This may be modified by covering the mirror with a paper or cardboard sheet having a circular cut-out so as to limit the image to that part of the specimen being demonstrated. Where numerous specimens are available, mirrors may be cut into suitable lengths so as to form a continuous mirror shelf. When set up with neat cardboard folders (11 by 14 inches) containing brief summaries of the clinical

*Received for publication August 13, 1931.

and pathologic aspects of the case as well as gross specimens and photomicrographs the whole makes an impressive and very instructive exhibit. The source of illumination is from above, the ordinary pendant or gooseneck brackets being satisfactory. The image is upright undistorted and of unusual clarity due to the

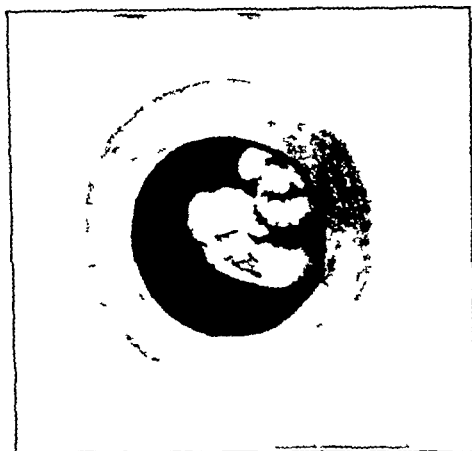


Fig. 2.—Photograph of mirror image seen through device as described. Photograph made with a 3x4 kodak one minute exposure 75 watt illumination using a magnified doubly reflected image. (Photograph has been retouched.) (Felsen J. The Use of a Magnified Doubly Reflected Image in Proctologic Demonstrations J. A. M. A. 9-23-1931)

presence of preserving fluid. There is no appreciable magnification where specimens are mounted in rectangular or square jars. The mirror-tube device being below the general level of the specimen shelf, neither obstructs a direct view of the museum jars nor interferes with the illumination of specimens from above. This has a decided advantage over a tube aimed directly at the pathologic area.

A MICROSCOPIC ARRANGEMENT FOR READING MACROSCOPIC KAHN PRECIPITATION TESTS*

By DAVID H. FLASHMAN, M.D., NEW KENSINGTON, PA

VARIOUS methods are in use for reading Kahn precipitation tests. One of the simplest is to use the inverted ocular eyepiece of a microscope. This method tends to result in scratching of the lens and does not give a high magnification. We have found the following method of using the compound microscope for this purpose to be such a decided advantage as to warrant its report.

The stage of the microscope is tilted at an angle of about 45 degrees. A simple wooden frame is constructed to support the small test tubes used in the test and to fit into the mechanical stage of the microscope. A mechanical stage is not necessary, but is very handy. Different types of mechanical stages will require different types of supports to fit them. We use a support composed of 2 side pieces of wood, each 4.8 by 1.2 by 0.4 cm., and of a baseboard 3.8 by 1.2 by 0.4 cm. The baseboard fits by friction into a space at the lower inner surface of our mechanical stage (Bausch and Lomb). The 2 side pieces of wood are nailed perpendicularly to the baseboard, so that the narrow sides rest semicircularly on the stage and the wide sides are parallel to each other about 13 mm. apart, to support a test tube 12 mm. diameter and about 72 mm. long. Fig. 1 illustrates how the test tube fits into the frame, as both lie on the stage of the microscope. The side pieces should be shorter than the test tube to leave room to grasp the tube.

The upper surface of the liquid in the tube remains horizontal to the ground, but is slanted in relation to the long axis of the tube. The condenser lens of the microscope is best removed and the diaphragm beneath the stage is much narrowed. The magnification is about 12 times, using a binocular microscope with 5X oculars and a number 3 Zeiss objective. If the lower lens of a 16 mm. objective (Bausch and Lomb) is removed, a similar magnification can be obtained. One focuses on the surface of the slanted portion of the fluid, at the thinnest region. Under these conditions, with the light satisfactorily cut down and the mirror properly adjusted, the lower zone of the microscopic field will show a bright light while the upper one-half or more will show a dark-field illumination, in which the particles of antigen can be seen as luminous points against a dark background.

It is helpful to shake the tubes gently before inserting them in the frame, as the moving of the particles allows more particles to be seen. If the tube is scratchy, one can rotate the tube in the holder in order to look through that portion of the tube most free from scratches. Some experience with the apparatus is of course necessary, but we have found that beginners acquire skill in reading the tests more quickly than with the use of an eyepiece lens.

*From the Pathological Laboratory of the Citizens General Hospital.
Received for publication, August 16, 1931.

The method permits greater sensitivity because the slightest degree of aggregation of the particles can be detected. The proportion of antigen and salt solution and the amount of antigen used naturally affect the dispersion of the particles as well as the sensitivity of the test. It is well to use that combination which gives with the antigen control and the negative sera a uniform, fine dispersion. With strongly positive sera, the clumps are large and few, and all degrees between this picture and that of the negative sera will be found. The method avoids mistakes due to foreign particles such as red blood corpuscles, clumps of bacteria, etc., because the fine particles of antigen will still be readily seen if the serum is negative, but if positive there will be a uniform aggregation of the fine particles to larger ones. It is the aggregation of the finer particles that indicates most definitely a positive reaction rather than the presence of larger particles. The results can be expressed as plus-minus, one-plus, two-plus, three-plus, and four-plus, depending on the degree of reaction.

With this method the results are simpler by using only one amount of

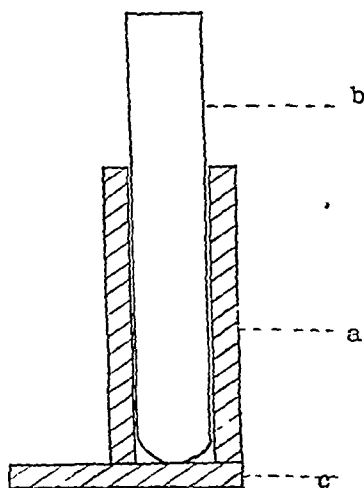


Fig. 1—Support for test tube. (a) Side-piece to support test tube. (b) (c) baseboard that fits into groove of mechanical stage. (Slightly reduced from natural size.)

antigen, instead of three as in the regular Kahn test, and the more sensitive quantity should be used. With the Kahn antigen sold by Parke, Davis & Co., we have found a 1 to 1.5 dilution of the antigen better than the 1 to 1 dilution recommended by the company. Also the use of 0.005 c.c. of this antigen dilution gave better results than the use of larger quantities. It is well also to use two different preparations of antigen, each adjusted to the most sensitive combination, as some serums react better with one antigen than with another. In this case the unknown sera tested with a given antigen must be compared with known negative sera tested with the same antigen.

Since the method promotes greater sensitivity by detecting the slightest degrees of reaction, more care must be used in the interpretation of these slight reactions. Inaccurate pipetting, unclean glassware, and other such sources of error would naturally be more apt to produce false slight reactions the more sensitive the method. Also the presence of other diseases would act similarly.

However, for treated cases of syphilis and as a supplement to clinical evidence, the slight reactions are helpful. One must bear in mind, however, that the less the degree of reaction, the lower the probability of the reaction indicating the presence of syphilis.

SUMMARY

A simple method is described for adapting the compound microscope for the reading of macroscopic Kahn precipitation tests. The stage of the microscope is tilted to an angle of about 45 degrees and one looks through the slanted portion of the fluid in the test tube, with the illumination cut down. A dark-field illumination of the particles of antigen is thus produced, giving a sensitive and easy method for reading the tests.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFF, M.D., ABSTRACT EDITOR

BILIRUBINURIA Methylene Blue Test for, Siede, J., and Zink, K. *Deutsche med Wchnschr* 57 1744, 1931

To 5 cc of urine add, drop by drop a 0.2 per cent aqueous solution of methylene blue. A blue color on the addition of the first drop indicates a negative reaction.

In the presence of bilirubin a green color appears. The number of drops of methylene blue required to change the blue color to green indicates the amount of bilirubin present (2 drops = 0.1 mg of bilirubin).

The following scale is suggested:

More than 10 drops strongly positive

2 to 10 drops weakly positive

B. TUBERCULOSIS Demonstration of Rare Tubercle Bacilli in Sputum, Pottenger, J. E. *Am Rev Tuberc* 24 583, 1931

The following method is advocated:

1 For collection of specimens and also for carrying out the technic use 8 ounce, wide mouthed, graduated bottles which are selected to fit the shaker. They are graduated for every 10 cc through paraffine with hydrofluoric acid. No. 16 cork stoppers, to fit the bottles, must be used once only.

2 Siphons, 3/4 mm lumen, for removing subnatant liquid.

3 Capillary pipettes with rubber bulb, for removing hydrocarbon layer in making preparation.

4 Open water bath for digestion, kept at 55° to 58°.

5 Closed flat top oven, containing water at 65° for drying films on slides, and for staining.

6 Mechanical shaker. The 4 bottle shaker manufactured by the International Equipment Co. has served well.

PROCEDURE FOR CARRYING OUT TECHNIC

1 Collect twenty-four hour or three day specimen.

2 Add equal parts of 0.5 per cent NaOH. Shake by machine for five to ten minutes.

3 Digest in water bath 55° for thirty minutes to one hour.

4 Pipette off and discard any insoluble residue which has settled at the bottom of the bottle.

5 Add 1 to 2 cc hydrocarbon (xylol, ligroin, benzene, or gasoline) fill with distilled water to about 200 cc. Shake ten minutes.

6 Allow hydrocarbon to collect at top of bottle (ten to twenty minutes).

7 If viscosity of specimen is too high, remove subnatant liquid by use of sterile siphon, fill to 200 cc again with distilled water, shake five minutes.

8 When viscosity is practically that of water the supernatant hydrocarbon layer is drawn up in a sterile pipette which is pinched by inserting cork and allowed to remain in vertical position until subnatant liquid separates from the hydrocarbon layer (five to ten minutes). The liquid is discarded and preparations are made from the hydrocarbon layer which is put on the slide and dried layer upon layer, to desired thickness.

9 Wash preparation in ether to remove traces of hydrocarbon and fat.

10 Stain preparation as usual. In decolorizing with alcohol, do not allow more than twenty seconds' exposure as brilliant decolorize rapidly. Complete decolorizing with 5 to 10 per cent sodium sulphate if necessary. Counterstain with 1 per cent aqueous picric acid.

PNEUMONIA Immune Transfusion In Lobar, Barach, I L, and Soroka, M Am J M Sc 182 81, 1931

Of 8 patients who were treated by immune transfusion, 6 were due to pneumococcus Type III and 2 to pneumococcus Type II. Four of the 6 patients of Type III cases had a bacteriemia and all died. The 2 patients of Type III cases with sterile blood culture survived. Of the 2 patients of Type II pneumonia with positive blood culture, 1 died and 1 survived.

The immediate effect of the transfusion on the clinical condition of the patient was negligible. No lessening of toxemia, prostration, cyanosis, or dyspnea was observed. In four fatal cases, the blood culture was not rendered sterile, nor was the spread of pulmonary consolidation checked. The determination of protective substance in the patient's blood before and after transfusion of immune blood showed an absence of the introduced protective substance in all the 5 fatal cases. Of the 3 who survived, 1 showed no protective substance in his serum until two days following the last transfusion, which therefore could not be ascribed to the introduction of immune blood. In 2 cases of pneumococcus Type III infection with sterile blood culture, the patient's serum contained protective substance after immune transfusion although not before. Their clinical course, however, was so long drawn out that it would not be possible to ascribe recovery to the protective substances introduced by transfusions.

Eight patients with pneumococcus lobar pneumonia, 6 due to Type III and 2 due to pneumococcus Type II, were treated by transfusion of 1000 to 1600 cc of blood from donors immunized over long periods of time.

No favorable effect was observed on the clinical condition of the patient as a result of any of the transfusions. The introduction of protective substance present in the donor's blood could not be demonstrated in the patient's blood in the 5 fatal cases and appeared to have no influence on the course of the disease in the 3 recovered patients.

TUBERCULOSIS Direct Culture In Tuberculous Effusions, Bezancon, F, and Buc, E Presse med 39 1493, 1931

The authors have had success in the culture of tubercle bacilli using the following medium: Peptone 2 gm, glycerin 5 gm, water 100 cc, neutralizing with 1 gm of monobasic potassium or sodium phosphate. The medium was tubed (2 to 3 cc per tube) and sterilized at from 110 to 115 C. Pleural fluid was inoculated in quantity almost equal to the tubed medium and the two liquids were mixed. Growth in the liquid usually appeared the third or fourth week in the form of cotton filaments, sometimes at the bottom of the tube as small opaque white granules, these increased in volume and assumed an arborescent aspect. The growth was more vigorous close to the surface of the liquid, there assuming cottony colonies. The bacilli were acid resistant, often highly granular, sometimes fragmentary, and virulent for the guinea pig.

In old thick, puriform, pleural effusions the microscopic examination was sufficient and animal inoculation was usually effective. For direct culture it was found necessary to add to the peptone glycerin medium 3 per cent gelose and one third volume of blood serum. In making cultures of old puriform exudates, as in serous exudates, inoculations were made exclusively from effusions in the course of artificial pneumothorax.

NEUROSYPHILIS Malarial Therapy of Other Than Uncomplicated Dementia Paralytica, Wile, U J, and Davenport, K M J A M A 97 1579, 1931

The authors thus summarize their experience since 1927:

1 Malarial therapy was used in the treatment of tabes, of dementia paralytica with tabes, and of diffuse neurosyphilis, including cases complicating the secondary stage.

2 In a large percentage of cases, immediate improvement was noted. Thus, 53 per cent of the tabetic group showed immediate improvement, and later observation increased this group to 67 per cent. In the greater number of these, improvement amounted to complete symptomatic remission. One patient showing immediate improvement relapsed later.

3 In patients with dementia paralytica with tabes, 40 per cent showed immediate symptomatic improvement, later observation increased this figure to 67 per cent. Thirteen

per cent of the cases were arrested, 13 per cent were made worse and 7 per cent of the patients died after leaving the hospital

4 The immediate results were most striking in the diffuse central nervous system group. In the group complicating secondary syphilis, all eight were immediately improved, seven later remained asymptomatic and one subsequently relapsed. In this case, recommended therapy was not carried out.

5 Twenty-four cases of later occurring diffuse neurosyphilis showed striking immediate improvement in all but one case. The later follow up of this group showed improvement in 84 per cent, no change in 10 per cent and a change for the worse in 6 per cent.

6 Ultimate gain in weight was an almost uniform feature, even in patients who did not otherwise improve.

7 Following treatment, many colloidal gold curves became negative, reversed, or became more or less intense without paralleling clinical results.

8 Reversal of the serologic reaction or diminution of its positivity occurred more often in the spinal fluid than in the blood in the group studied.

9 Decrease in cell counts and organic solids was almost invariably noted.

10 From the foregoing conclusions, it is apparent that malarial treatment is a definitely beneficial addition to the armamentarium of neurosyphilitic therapy.

B. TUBERCULOSIS. A Potato-Egg Medium for Isolation, Woolley, J. S., and Peter, F. G. *Am. Rev. Tuberc.* 24: 596, 1931.

Preparation of Medium—

All glassware is sterilized in the autoclave, also egg beater, potato masher, etc. Eggs should be soaked in alcohol (70 per cent). The culture tubes, 6 × 7/4 inches, are stoppered with a No. 8 long cork stopper and heated in a hot air oven at 275° F. for one and one-half hours. The use of the cork stopper helps to retain the moisture during sterilization and later. It also facilitates the seeding of the tubes as the stoppers can be easily withdrawn.

Autoclave medium sized unpeeled potatoes for thirty minutes at 15 pounds' pressure. Peel, and after thorough mashing, suspend in 15 per cent glycerol water, in the proportion of 500 gm. of original potato to 500 cc. of 15 per cent glycerin. Heat in a boiling water bath for thirty minutes, stirring occasionally. Strain through two layers of surgical gauze by compression into a flask and then boil filtrate for about five minutes. Cool and add one part of filtrate to two parts of well beaten whole fresh egg. Add sufficient 1 per cent crystal violet to make a concentration of 1 to 30,000. Stir thoroughly, to distribute the dye evenly throughout the mixture. Pour the mixture through one layer of sterile gauze into a tubing funnel and add approximately 7 or 8 cc. of medium to each tube. Sterilize in the inspissator at the proper slant for one-half hour at 85° C. on the first day, and at 75° on the second day. Bring inspissator up to required temperature before introducing tubes. By omitting the crystal violet an excellent medium is obtained for growing stock cultures of tubercle bacilli. Incubate the tubes to test for sterility. Tubes must be stored, seeded and incubated in an upright position to keep the surface of the medium dry.

The sputum before isolation was treated as originally suggested by Corper.

One cubic centimeter of sputum is beaten into a homogeneous pulp and introduced into a sterile 15 cc. centrifuge tube with 1 cc. of "6 per cent sulphuric acid" (prepared by adding 17 cc. of sulphuric acid, specific gravity 1.84, to distilled water of 500 cc. final volume). After thorough mixing the tube is stoppered with a sterile cork and incubated at 37° C. for thirty minutes. During this period it is occasionally shaken. The contents are then diluted with about 10 cc. of sterile physiologic sodium chloride solution, well mixed and centrifugated. The supernatant fluid is decanted, and the residue cultured or inoculated without further treatment.

MENINGITIS. The Diagnostic Value of Smears from Purpuric Lesions in Meningococcus Bacteremia, McLean, S., and Caffey, J. *Am. J. Dis. Child.* 42: 1053, 1931.

The following procedure was found to be of early diagnostic value in endemic purpuric meningococcus infections.

The technic of examining purpuric skin lesions for meningococci is simple. The skin surface over the cutaneous lesion is cleansed with alcohol and permitted to dry, and then a small stab wound is made with a Hagedorn needle. Usually, the largest lesions are selected for puncture. The examination, however, was satisfactorily completed several times with small petechiae. After the skin puncture, the extravasated blood and tissue juices from the lesion are smeared onto a slide or cover glass. They are then dried in the air and stained by Gram's method. Microscopic examinations are then made with the high power oil immersion lens. The material on the preparation varies considerably in its content of blood cells and polymorphonuclear leucocytes. Usually a surprisingly large number of the latter cells are present. Search is made until typical intracellular organisms are identified. In negative cases search was continued for at least one hour before a negative result was tabulated.

The results are positive only when the gram negative diplococcus has been seen within polymorphonuclear leucocytes. Typical looking organisms were frequently present extracellularly as well as within the cells.

POLIOMYELITIS The Spinal Fluid Cytology, Thelander, H. E., Shaw, E. B., and Lemper, M. A. *Am J Dis Child* 42: 1117, 1931

In a series of 122 cases of poliomyelitis, the cell count of the spinal fluid varied from 10 to 700, with the greatest number of cases between 50 and 200 and about an equal number below 50 and between 200 and 300, dropping off rapidly over 300, but a few scattered cases occurring up to 700. A high percentage of cases of bulbar involvement may account for the large group with a low cell count.

About one half of the cases had a polymorphonuclear percentage over 50, the peak of the curve being in the group from 50 to 75 per cent. This finding is not in accordance with most authors and may be accounted for by variations in different epidemics and by the technic of staining and studying the cells.

The percentage of polymorphonuclears during the acute phase of the disease was independent of the day of the disease. The presence of a high percentage of polymorphonuclears is dependent, therefore, probably on some factor other than the stage of the disease.

The blood count quite consistently showed a slight leucocytosis with a relatively high polymorphonuclear count.

ARTHRITIS DEFORMANS Role of Streptococcus in An Improved Cultural Method, Gray, J. W. and Gowen, C. H. *Am J M Sc* 182: 682, 1931

The following modification of Cecil's technic is said to show growth in one to four days.

The patient's arm is prepared by two coats of iodine, washed off with alcohol and 20 cc of blood is drawn from a vein in the arm, and placed in two sterile dry test tubes (10 cc in each). These are placed in the ice box over night. The serum is removed, clot broken up, and the pieces of clot placed in each of two 100 cc bottles containing 50 cc of media. The media is prepared as follows: Fresh beef heart is freed from fat and fibers, ground finely in a meat chopper and infused at ice box temperature over night, using 500 gm ground meat and 500 cc of tap water. The next morning the infusion is warmed from 20° to 25° C and squeezed through a flannel bag. The filtrate is then boiled slowly for one hour and filtered through paper. It is then made up to volume, 1.5 per cent peptone (Witte), 0.5 per cent chloride of sodium, 1 per cent dextrose, and 1 per cent gelatin added. This is then placed in the Arnold sterilizer for twenty to twenty five minutes to dissolve the ingredients. It is then titrated to PH 8 and placed in the Arnold sterilizer for one hour. It is filtered through paper and retitrated. If the PH has dropped below 7.8 it should be retitrated to that figure. It should not be below a PH of 7.8 before placing in bottles. The bottles are prepared beforehand by placing about a teaspoonful of calcium carbonate (cp, powdered) in each of them, plugging with cotton, or cheesecloth, and cotton and sterilizing in the dry sterilizer for one hour. In these sterile bottles 50 cc of medium are placed and sterilized in the Arnold sterilizer for thirty minutes on three successive days.

At the end of three days it is titrated and if the PH is 7.6 to 7.8 it is satisfactory. It usually shows a PH of 7.7 to 7.8. If the PH is correct, the medium is placed in the incubator for several days and if sterile is then ready for use.

The authors thus summarize their investigation:

1 Our results confirm recent investigation that arthritis deformans is due to an Alpha type streptococcal infection of the joints.

2 Climate and fatigue are important predisposing factors.

3 The Alpha type or Alpha prime streptococcus causing arthritis deformans produces slight hemolysis in primary cultures.

4 The blood or joint fluid was positive for this streptococcus in 62 per cent of 71 arthritis deformans cases.

5 An improved cultural method for the quick growth of this organism from the blood and joint fluid is described.

6 The clinical picture and pathology of arthritis deformans are typical of an infectious process.

7 Agglutination tests are of considerable value in diagnosis.

8 The importance of general and focal treatment should not be underestimated.

9 Specific vaccine therapy is the most efficient form of treatment because it has cured or improved patients who were becoming progressively worse following other forms of treatment.

10 Vaccine should be prepared from blood or joint cultures when possible, otherwise from cultures from foci or from stock specific strains.

11 Vaccine treatment preliminary to the removal of foci, particularly badly infected tonsils, might prevent undesirable joint reactions.

12 Vaccine for the cure of joint infection must be continued for a long period of time.

13 Intravenous vaccine promptly relieves symptoms and probably controls the joint infection more quickly than subcutaneous injection.

14 The dose of vaccine and interval between injections should be so regulated that reactions do not occur, particularly joint reactions.

TISSUE Method for Examination of the Appendix, Steinberg, B Arch Path 12 598, 1931

The method below is useful in determination of a perforation and the localization of the lesion.

An ordinary 5 or 10 c.c. syringe is partly filled with a weak solution of eosin. A needle is attached to the syringe, and the needle is introduced into the lumen of the appendix through its proximal end. A hemostat is applied over the appendix and needle to keep the needle in place and to prevent the escape of the eosin. The hemostat is applied over that part of the appendix which shows the hemostat markings made by the surgeon. The piston of the syringe is gently pushed down so that the eosin solution runs into the appendiceal lumen. At the point of perforation, the eosin escapes through the wall and marks the point of the perforation. This method of filling the appendix was found preferable to the introduction of the fluid by gravity. The slight pressure exerted was not found to produce artificial perforations in gangrenous appendices. If a permanent record is desired of the perforation and its location, iodized poppy seed oil 40 per cent may be introduced instead of eosin and a roentgenogram taken.

The following method is suggested for cutting and sectioning the appendix.

After the appendix is received from the operating room it is placed in a 10 per cent formaldehyde solution for from six to twenty-four hours. The organ is then removed from the fixative and cut longitudinally with a long and flat bladed knife. The cut is begun at the tip of the appendix with the heel of the knife and carried longitudinally through the approximate middle of the organ. The appendix is supported gently with the left hand, and the knife is carried in a single cut to avoid an irregular surface. At the completion of the section two equal halves are obtained. Each half of the organ shows the lumen, its contents and the wall.

For histologic sections one or both halves may be used. Any method of handling the tissue preparatory to embedding may be employed. However, Sternberg believes it preferable to use alcohol for dehydration and chloroform for clearing. Either celloidin or Warthin's celloidin sheet method or paraffin embedding may be selected. The paraffin method requires more skill and care in preventing wrinkles and tears. Sections 6 microns in width are made through various levels in which the appendical lumen persists. From five to ten sections will give a composite picture of the entire organ. The sections allow the determination of the width of the lumen and the relation of the opposing surfaces, and make it possible to study the whole length of the appendix in a single or two sections.

GLANDULAR FEVER Protozoal Nature of The Experimental Disease, Bland, J O W
Brit J Exper Path 12 311, 1931

The results of this study are thus summarized

1 The rabbit diseases, previously called "experimental glandular fever," which followed the inoculation of blood from two cases of human glandular fever is caused by protozoa of the genus *Toxoplasma*

2 These protozoa closely resemble the *Toxoplasma cuniculi* and are immunologically identical with it. The disease they cause is indistinguishable from that produced by a strain of *T. cuniculi*

3 The protozoa differ from ordinary *T. cuniculi* in their greater virulence for rabbits and in their power to infect monkeys in which animal they produce a disease very like human glandular fever

4 No similar disease has been produced in rabbits of the same stock by control inoculations with normal rabbit or normal human blood or with blood from febrile people

5 The evidence suggests that human glandular fever may be caused by the protozoa described, but this requires confirmation

DAKIN'S SOLUTION Simple Test for Available Chlorine Strength, Etc., Ullrich, A. H
Am J Pub Health 21 1237, 1931

Apparatus —

Pipette graduated to deliver 5 c.c. Dakin's or equivalent chlorine solution (Note: Our test is based on the use of a 5 c.c. sample of the solution to be tested.)

Pipette with two graduations, the first to deliver 6.43 c.c. N/10 AsO₃, the other to deliver 7.86 c.c. N/10 AsO₃.

Two 2 ounce ground glass stoppered bottles to be marked No. 1 and No. 2 respectively for convenience

1 c.c. medicine dropper for orthotolidin

Reagents —

Standardized N/10 AsO₃ solution

Standard orthotolidin solution

Procedure —

To each of the 2 ounce bottles add approximately 20 c.c. of distilled water and then exactly 5 c.c. of the solution to be tested

To bottle No. 1 add standard arsenious acid solution as measured by the lower mark on the graduated pipette or 6.43 c.c.

To bottle No. 2 add standard arsenious acid solution as measured by the upper mark on the graduated pipette or 7.86 c.c.

Shake contents of both bottles

Add 1 c.c. orthotolidin solution to each bottle

Results —

No color in either bottle, solution tested weaker than the accepted Dakin's solution

Yellow to orange color in bottle No. 1 but no color in bottle No. 2, solution tested has correct strength for accepted Dakin's Solution

Color in both bottles, solution tested is stronger than accepted Dakin's solution

SPINAL FLUID A New Test, Gruskin, B. Am J Clin Path 1 441, 1931

Ten Wassermann tubes are set up in a rack. Into Tube 1 there is pipetted 0.3 c.c. of spinal fluid, into Tube 2 there is pipetted 0.23 c.c. of spinal fluid. To Tube 1 there is added

0.7 cc of physiologic saline, and to Tube 2 is added 0.77 cc of saline, bringing the volume in each tube up to 1.0 cc. Into each of the other eight tubes 0.5 cc of saline is added. The fluid in Tube 1 is well mixed by gently shaking or by pipetting and 0.5 cc is transferred to Tube 3 mixing well and transferring 0.5 cc to Tube 5, and so on in alternate tubes until the ninth tube is reached, from this tube 0.5 cc is discarded. Then beginning with Tube 2, the fluid is well mixed and transferred similarly in series to Tubes 4, 6, 8, 10, discarding the 0.5 cc from Tube 10.

To each tube is then added 1.0 cc of starch iodine solution. The tubes are carefully agitated until the color is uniform in each tube, and the color of each tube is read immediately. The final readings are taken one half hour later.

The starch iodine solution is made as follows:

One part aqueous iodine (0.1 gram in 1000 cc distilled water)

One part starch solution (0.75 gram in 100 cc of saline)

One part physiologic saline (8.5 grams NaCl in 1000 cc of distilled water)

One tenth gram of fine iodine crystals will dissolve in the water in from three to four weeks. The water must be absolutely free from organic matter so that the solution of iodine will not be weakened by any reaction or standing. Soluble starch is used for the starch solution and is heated only until a clear solution is obtained. The solution need not be boiled and should not be used when it has become cloudy on standing. The glass ware for the test should be chemically clean and dried by sterilizing in the gas oven, thus keeping it free from foreign matter, such as lint, dust and the like.

The readings are made in symbols as follows: decolorized tube, O, light blue color, L, standard blue of starch iodine solution, B. Enclosing the letter in parentheses indicates a lesser value, thus, (L) indicates a very pale blue color and (B) a slightly affected standard blue color.

In normal fluid two tubes are slightly affected and one tube is sometimes decolorized.

In tabes, from four to five tubes are affected and two of these are decolorized.

In paresis, from five to six tubes are affected and three of these are decolorized.

In meningitis, from seven to nine tubes may be affected, according to the severity of the case and from five to six of these are decolorized.

PHOTOGRAPHY X-ray Ink, Raison, T. W. Radiographs 7 13, 1931

The following formula, devised for marking x ray films, conceivably might be used for marking microphotographs:

Water	20 cc
Sodium iodide	11 gm
Barium sulphate	40 gm.
Mucilage of Acacia	2 gm
Chloroform	1 cc

The sodium iodine content can be varied from the amount stated in the formula, more iodide gives greater x ray absorption, although poorer writing and drying qualities, while the reverse is true for lesser quantities of iodide.

Since the barium sulphate tends to settle on standing, the ink should be well shaken before using. A ball pointed pen is recommended for use with this ink, and the writing should be done rather slowly in order to produce a broad, heavy line.

POLIOMYELITIS Results of Treatment in One Hundred and Four Cases, Shaw, E. B., Thelander, H. E., and Lemper, M. A. J. A. M. A. 97 1620, 1931

The study is thus summarized:

One hundred and four patients with poliomyelitis were admitted to Children's Hospital in the period from July 1 to Dec 31, 1930. Specific therapy was attempted in 92 cases.

Of 53 patients treated before the onset of paralysis, 28 showed no paralysis at any time, 15 showed transient weakness which had entirely disappeared before dismissal, 9 showed persistent paralysis and one died.

The average age of the unparalyzed patients was nine and one half years, of the transiently paralyzed, 10 years, and of those with definite paralysis, 17 years. This is at least significant to the hypothesis that better results are obtained in the lower age group.

The average spinal fluid cell count was 146 in the unparalyzed group, 119 in the transiently paralyzed group, 197 in the persistently paralyzed group, and 270 in the single fatality. These averages were made up from widely varying individual cases and, we believe, are without significance.

Serum was applied, on the average, 27 days after onset of symptoms in the unparalyzed groups, 36 days in those with transient paralysis, and 34 days in those with persistent paralysis.

The average amount of serum used in the group treated preparalytically was, respectively, 120, 151, 209, and 375 c c.

In the group treated in the acute stage after the appearance of demonstrable weakness, of 39 patients, 9 showed transient weakness, 23 had persistent paralysis, and 7 died.

The average age in those with transient weakness was 6.8 years, 11.7 years in those with persistent paralysis, and 19.8 years in the fatal cases, again showing the higher average age in those with serious outcome.

Average cell counts were 67 in the transiently paralyzed, 194 in those with persistent paralysis and 199 in the fatal cases.

Treatment was instituted on the average of 37 days after onset of symptoms in the transiently paralyzed, 42 days in the cases of paralysis and 63 days in the fatal cases, coinciding with the general idea of the importance of early treatment.

The transiently paralyzed patients received an average of 84 c c of serum or plasma, the permanently paralyzed 156 c c and in the fatal cases, 156 c c was given.

Of the 53 patients treated before the onset of paralysis, 83.4 per cent showed no permanent paralysis, 16.9 per cent showed definite persistent paralysis, and 1.9 per cent died. Of the thirty nine patients treated after the onset of paralysis while the disease was still acute, 23.08 per cent showed no end paralysis, 59 per cent showed definite paralysis and 18 per cent died. It is unfair to attempt to compare the results in these two groups of cases. While the first group included at least a few benign cases, the second group included many extremely virulent cases referred to the hospital because of their fulminant course.

MULTIPLE SCLEROSIS Study of the Etiology of Weil, A. J. J. A. M. A. 97 1587, 1931

Having first learned their methods by working with Chevassut and Purves Stewart the author repeated their work on the etiology of multiple sclerosis with the following results:

- 1 Repetition of the experiments of Chevassut and Purves Stewart failed to produce convincing evidence that, in multiple sclerosis, cultures from spinal fluids yield a filtrable virus and that this virus is responsible for the production of the disease.

- 2 The fact that spheres and colonies of spheres may more readily be seen in agar cultures of spinal fluids that have given a positive globulin reaction suggests the precipitation of colloidal protein (or lipid) particles, which become visible in the dark field.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor,
Dr Warren T Vaughan, Professional Building, Richmond, Va

Klinische Laboratoriumstechnik

THIS third volume consists mostly of functional tests of various organs such as the liver, kidneys, pancreas, stomach, intestines, and lungs, the gall bladder and extrahepatic bile ducts, the heart, blood vessels and the circulatory system as a whole, and the organs of internal secretion such as the thyroid, hypophysis, adrenals, gonads and pancreas. There are also chapters on capillaroscopy, vital staining, in vitro cultivation of tissues, serologic diagnosis of cancer, and methods of testing the physiological effects of athletic training. A large number of tests are given under each heading and for each test not only are the apparatus used and the methods employed fully described but a critical evaluation is made of each test and a full bibliography added.

Die Regulierung der Atmung†

THIS monograph is a companion volume to the author's "Regulierung des Blutlaufes" (Thieme, 1930). It consists of a thorough discussion of respiratory phenomena. The action of such chemical agents as carbon dioxide, oxygen, acetylcholine, pituitrin, epinephrine, etc. are discussed and their effects correlated with circulatory, nervous, and mechanical factors. The author also offers new evidence concerning the Hering-Breuer reflex, in which he lays much stress upon the part played by the diaphragm. A bibliography of over 400 titles is affixed.

Die speziellen Blutkrankheiten im Lichte der qualitativen Blutlehre‡

IN this second volume the author considers in detail the blood pictures encountered in various pathological conditions, grouping them according to the type of blood reaction produced.

He devotes 90 pages to agranulocytic reactions, 90 to lymphocytic reactions, 150 to lymphatic leukemic reactions and the rest of the book to the lesser reactions. His method of differential analysis of blood cells resembles the better known method of von Schilling but differs from it in many details.

*Klinische Laboratoriumstechnik (Clinical Laboratory Methods) By Brugsch and Schittenhelm. Vol. III. Published by Urban and Schwarzenberg, Berlin and Vienna, 1928.

†Die Regulierung der Atmung (The Regulation of Breathing) By W. P. Hess. Georg Thieme, Leipzig, 1931. 137 pages.

‡Die speziellen Blutkrankheiten im Lichte der qualitativen Blutlehre (Special blood diseases in the light of qualitative blood examinations) By Joseph Arneth. H. Stenderhoff, Münster, 1930.

NOTE. In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

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EDITORIAL

Experimental Studies of Dengue

TIME was when diseases could be labeled geographically, as it were, and spoken of as "tropical," "Oriental" and the like with some confidence that necessity for any familiarity with their characteristics rested mainly upon physicians practicing in appropriate localities

The changes brought by modern methods of rapid transportation, however, have almost erased the geographic boundaries of disease and to that extent have complicated and extended the scope of the diagnostic problems in which the practice of medicine abounds

For these reasons, as well as for the interest which attaches to the clarification of any puzzling problem in medicine, the recent comprehensive report upon studies in dengue by the United States Army Medical Department Research Board constitutes a contribution of distinct importance¹

Dengue, or "breakbone" fever, is an acute disease characterized by sudden onset, initial erythema, headache, and pains in the trunk and limbs, especially

¹Simmons J S St John J H and Reynolds F H K. Experimental Studies of Dengue
Philippine J Sc 44 1 1931

the joints, the pain and stiffness in which lead to a peculiarity in gait from which the term "dandy fever" arises

The disease has long been the subject of experimental investigation

That the etiologic agent of dengue is a filterable virus which can be demonstrated in the circulating blood and that the disease may be transmitted by the mosquito (*Aedes aegypti*) was first shown by Ashburn and Craig in 1907, their results being corroborated by the later work of Siler, Hall, and Hitchens in 1922

The investigations thus begun have been continued and extended by the authors of the present report whose studies were planned to consider

- a The epidemiology of dengue
- b The nature of the virus
- c The possibility of transmitting the disease by insects other than *A. aegypti*
- d The possibility of transmission of dengue from infected to normal insects
- e The search for a susceptible experimental animal
- f Search for improved diagnostic methods
- g Observations on the therapeutic value of immune sera
- h Investigations of a prophylactic vaccine

While all of these problems have not been solved, the investigation has definitely added to the present knowledge of dengue and will further the progress of future investigations not only of dengue but also of yellow fever, as the two diseases, though differing greatly in severity, as dengue is practically never fatal, nevertheless have many points of similarity

1 *The Epidemiology of Dengue*—The present studies indicate that, as far as the Philippine Islands are concerned, dengue occurs in seasonal waves reaching their peak sometime between April and November. There is a direct relation between the monthly rainfall, and hence the mosquito prevalence, and the prevalence of dengue. The suggestion is advanced that the dengue virus may be maintained in an endemic area by transfer through (a) susceptible native children, (b) incompletely immunized native adults (c) susceptible newcomers from dengue-free localities, and possibly, (d) by direct transfer from infected to normal insect vectors

2 *The Transmission of Dengue by Aedes Aegypti*—The importance of this mosquito as a dengue vector is again substantiated by the present investigation which has added to the understanding of the mechanism involved

It appears definitely demonstrated that the mosquitoes become infected only when they feed during the first forty-eight hours of the disease, that the disease may be transmitted by small lots of five or ten just as consistently as by large groups of one hundred to one hundred and fifty, and that infectivity persists for from sixty to seventy days, if not indefinitely at the temperatures prevailing in Manila (maximum 36.7°C, mean, 26.3°C, and minimum 17.4°C)

3 *The Transmission of Dengue by Insects Other Than A. Aegypti*—The investigations described in the report offer positive proof that not only *A. aegypti* but also *A. albopictus* transmit dengue and hence suggest that it is reasonable to suspect that still other members of the *Aedes* species may be concerned in the spread of this disease. Experiments with *Culex quinquefasciatus* indicate that

if this mosquito is able to transmit dengue to man, this probably occurs only as a result of mechanical transfer of blood during interrupted feeding. That is, a *C. quinquefasciatus*, interrupted in its feeding upon a dengue-infected individual, when it resumes feeding upon a noninfected individual, may mechanically transmit the virus. While such mechanical transmission of the disease has been proved experimentally, relatively large numbers of mosquitoes are necessary for such transmission, and it is probable that it occurs but rarely in nature.

Contamination of the skin by crushed *A. aegypti* or other insects in which the virus might exist does not appear to be a factor in the natural spread of dengue.

The conclusions of Siler, Hall, and Hitchens that the virus of dengue is not transmitted from infected female *A. aegypti* through egg to offspring are corroborated by the present commission, nor was it possible to infect mosquito larvae with the dengue virus, although it was not possible to be certain that the dengue virus added to the water was ingested by these larvae or that it remained alive long enough to cause infection.

As dengue virus escapes from the proboscis of infective female *A. aegypti* while they are feeding on blood, it was considered possible that a similar escape of the virus might occur during the ingestion of other foods or water and thus make it possible for normal mosquitoes feeding subsequently on the contaminated material to become infected. While this possibility was not proved experimentally, the evidence was suggestive and the contingency cannot be dismissed, particularly as in other experiments it was shown that the virus of dengue could be transferred by feeding normal *A. aegypti* on infected mosquitoes macerated and suspended in normal blood.

Under these circumstances not less than five infective mosquitoes per cubic centimeter of blood were required, a period of more than seven days being required before mosquitoes thus infected could transmit the disease.

4 *The Nature of the Virus*—Attempts to demonstrate the dengue virus were entirely unsuccessful although attempted with various ways.

The results of cultivation experiments were entirely in accord with those of previous investigators in suggesting that the virus of dengue was incapable of multiplication in the culture media used (Noguchi's serum medium, Boeck and Drbohlav's amoeba medium, Hibler's brain medium, MNN medium, brain agar, Frances' cystine agar, dextrose infusion broth, and mosquito broth under aerobic and anaerobic conditions at 37.5°C and room temperature).

From available information it was evident that the dengue virus remains alive in its mosquito and animal hosts for much longer periods than in the dead tissues of these hosts, although the duration of life in the latter under favorable conditions may not be inconsiderable. The virus was not preserved in an infective state in desiccated infective mosquitoes stored at 18°C for four days nor in frozen dried blood collected during the first day of fever.

The virus was again shown to be filterable during the course of the present experiments.

5 *The Clinical Aspects of Dengue Fever*—Based upon experimental cases the clinical aspects of the disease may thus be summarized.

The average incubation period was 5.66 days, duration of fever 4.8 days, with primary rash, 33.8 per cent, with secondary rash 69.5 per cent.

Additional symptoms commonly observed were leucopenia (100 per cent), postorbital pains and backache (90 per cent), altered sense of taste (65 per cent), pains in limbs (60 per cent), adenopathy (47 per cent), pains in the joints (43 per cent).

In 58 per cent of the cases (60) the onset was abrupt, usually with chill, while in 81 cases the disease was mild in 13.6 per cent, average in 70.4 per cent, and severe in 16 per cent.

From the diagnostic standpoint the leucopenia and other associated leucocytic signs were of the greatest diagnostic value, no significant changes being observed in the red cells or blood platelets.

Leucopenia was invariably encountered at some time during the infection, after its initial appearance was progressive, and often lasted for several days after the subsidence of the fever.

As a rule the leucopenia began on the second day, reaching a low point in the fourth, fifth, or sixth day after onset, and gradually returned to normal on the third or fourth day of convalescence.

Differential studies showed the leucopenia to be due to a decrease in both the lymphocytes and mature neutrophilic granulocytes, the former first returning to the normal level during convalescence.

Coincident with the appearance of the leucopenia there was an enormous increase in the number of immature granulocytes. This sharp "shift to the left" was a constant reaction and together with the leucopenia, constituted the most reliable single diagnostic sign of dengue fever.

6 Dengue Immunity—No evidence has yet been advanced to prove that all people of any race or group may be naturally immune to dengue. On the contrary, a large proportion of human beings are apparently naturally susceptible as is indicated by different epidemics involving from 75 to 100 per cent of inhabitants, particularly in places where the disease is first introduced.

That immunity is produced is shown by studies of adult natives living in endemic areas, the immunity in Filipinos being apparently well developed, although not necessarily invincible under experimental conditions.

The present studies suggest that the immunity following an attack of dengue fever in persons residing in endemic areas is probably completely protective in the majority of instances. There is some possibility, although this remains to be demonstrated, that the immunity produced by a single attack may be maintained by subsequent introduction of virus from infective mosquitoes.

Attempts, during the fever and following recovery, to demonstrate specific antibodies in the serum were not successful.

7 Prophylactic Vaccination—While only failure followed vaccination of volunteers with filtrates from saline suspensions of macerated infective mosquitoes, saline suspensions of noninfective immune dried blood, and a vaccine prepared from infective *A. aegypti*. Simmons, Reynolds and St. John believe that an effective prophylactic vaccine against dengue may yet be developed by further investigation.

8 *The Search for a Susceptible Experimental Animal*—Proof is offered that dengue can be transmitted to monkeys (*M. fuscatus* and *M. philippinensis*) by infected *A. aegypti*, that the virus multiplies in the blood without producing recognizable typical changes in the temperature or the leucocyte counts, that at some time between the third and ninth day the virus may be passed to other monkeys by blood inoculation or may be transferred through *Aedes* to other monkeys or man, and that in one monkey a single attack produced an immunity which lasted for several days

As, therefore, monkeys are able to take part in the natural dissemination and maintenance of the virus in endemic tropical localities they are of considerable importance to any consideration of the epidemiology of dengue

Attention is also called to the fact that negative conclusions should not be drawn concerning animal susceptibility unless (a) the animals tested are known to have been protected throughout their lives from the bites of infected mosquitoes, and (b) unless they are tested daily from the fourth to the eighth or ninth day after the introduction of the virus by transfer of blood, either directly or indirectly through mosquito vectors to susceptible volunteers

So closes a report representing a long and careful series of investigations no reference to which would be complete without mention of the volunteers whose willingness to become the subject of experiment made the investigation possible and in no small measure contributed to the measure of the success it attained

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EXPERIMENTAL ADRENAL EXHAUSTION*

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NUMEROUS researches have been carried on in the past few years upon the nervous control of the adrenal glands, upon the effect of various poisons upon the output of epinephrine and upon the store of epinephrine which remains in the glands following the action of the poison. The relation of the splanchnic nerves to the glands has also been extensively studied and the importance of these nerves in the control of the output of epinephrine has been repeatedly demonstrated. It has been shown that some of the poisons lead to a certain diminution in the amount of epinephrine in the gland and in fact hunger and cold may have a like effect.

Among the toxins which greatly lessen the epinephrine store, diphtheria toxin occupies a very prominent position. In this connection an important fact was pointed out by Elliott,¹ viz. that the action of this poison is an indirect one and exerted mainly through the central nervous system as its effect is wanting when the splanchnic nerves are cut.

Among the drugs which affect the epinephrine output physostigmine² pilocarpine³ and strychnine⁴ are perhaps the most important. These have been studied by Stewart and Rogoff who have demonstrated that there is a distinct increase in the epinephrine content of the blood following the administrations of these alkaloids. This increase is most marked when physostigmine is given. Certain other drugs are said to have a similar action but the effect is not nearly so pronounced as with those named. One of the less known drugs which apparently possesses this action is a tellurium compound studied by Cow and Dixon⁵ who

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ascribed its effect upon the circulation to the liberation of epinephrine from the adrenals. Section of the splanchnics did not alter the pressor effect of the drug so that it would seem to be due to a direct effect of the compound upon the glands themselves. Repeated injections of the compound tended to produce diminishing results which fact is ascribed to an exhaustion of the glands. A delay, however, before further injections sufficed to produce a normal response again. The rest interval necessary for recovery is not stated but it forms quite an important detail in giving some indication of the rate of epinephrine replacement in the gland. It might be questioned whether the failure of circulatory response to repeated injections is due to lack of available epinephrine or to fatigue of the structure acted upon due to too frequent stimulation. The importance of the point lies in the fact that the conclusion is drawn that during the brief rest interval epinephrine is replaced and that there seemed to be no limit to the amount which the gland can manufacture provided "a little time is given." It will be seen at once that these facts have an important bearing upon the general question of epinephrine replacement, a matter which we have studied in detail, as will be discussed later in this paper. The direct evidence which Cow and Dixon cite in support of this point is found in the epinephrine content of two glands, one removed before, the other after the animal had received six injections of the telonium compound. Very little difference was found indicating that the gland replenished itself very rapidly. It should be pointed out, however, that a difference in epinephrine content between the two glands would hardly be expected under the conditions described. The amount of active principle which is necessary to raise the blood pressure is so minute that even six stimuli from the telonium compound would call forth an almost negligible amount of epinephrine. This fact is supported by the results obtained by many investigators who after repeated stimuli of one splanchnic nerve found no lessening of the epinephrine content of the gland as compared with the resting gland.

For instance Stewart, Rogoff and Gibson⁶ stimulated the left splanchnic of a cat 52 times in 4 hours. At the end of the experiment the left adrenal yielded 0.14 mg. epinephrine while the right control gland contained 0.10 mg. The situation is much the same when certain drugs are injected which in themselves increase the epinephrine content of the blood but which may or may not affect the store of the active principle. Some lessen the store and others have no such influence.

As an illustration Stewart and Rogoff found that pilocarpine caused a very mild diminution in epinephrine store. In one cat to which 31 mg. had been administered within five hours, both adrenals being the same weight, one gland protected by cutting the splanchnic nerve contained 0.31 mg. of epinephrine while the gland with intact nerve supply had only 0.24 mg. The work of Elliott pointed to a smaller difference even than that quoted above.

Strychnine also was found to increase the epinephrine output even to five or six times the normal amount through a central action and yet examination of the glands on the two sides—one with intact splanchnic and the other with the nerve sectioned—showed no difference in epinephrine store. These cats had been subjected to the action of strychnine for many hours and had apparently been pour-

ing out epinephrine in increased amounts and yet with no diminution of store. The only conclusion to be drawn is that under the influence of the strychnine the animals must have been forming epinephrine in larger amounts to allow for the increased output without depletion of the glands.

Still another drug which has a marked effect upon epinephrine output is physostigmine. It has been shown that this alkaloid will increase the epinephrine content of the blood even to fifteen times the normal amount.²

Recently Crowden³ has shown that the adrenals can be partially depleted by subjecting the animal to external cold. Cats made wet were kept at a temperature of 0° C. for some hours and examination showed that there was a certain degree of depletion in most of the animals. Moreover the depletion was from 20 to 30 per cent greater in the normally innervated gland than it was in the gland with the nerve connection severed. Experiments were carried out also to study the rate of recovery of the glands after such depletion and it was found that while eighteen hours was insufficient three days seemed to be ample.

Our own experiments were carried out in an attempt to study further the phenomena of adrenal depletion and recovery—what factors might affect them and whether possibly any light might be shed upon the nature of the precursor for epinephrine in the body.

The first part of our study was similar in nature to that carried out by Crowden, viz., to ascertain what agent, if any, could be relied upon to produce epinephrine depletion. We studied first of all physostigmine inasmuch as it had apparently proved to be the most active adrenal stimulant. Our work was done exclusively on dogs and the method of extracting the epinephrine from the glands was essentially that described by Folin, Cannon, and Denis.⁴ Metz synthetic epinephrine was employed as a standard, a sufficient amount of N/10 HCl being added to the water containing the alkaloid to bring the latter into solution. A 1/1000 solution thus prepared was further diluted as needed for injection. We found the biologic method of estimating the strength of epinephrine solutions—by means of the blood pressure method on dogs—to be much more satisfactory than the colorimetric method so that we used it exclusively. The details of the manner of carrying out this method of assay are so well known that their repetition here is superfluous. As a control a total of eleven normal dogs gave an average content of 1.51 mg. of epinephrine for each gram of fresh gland. These figures were a little higher than those usually accepted as being normal for dogs, viz., 0.1 per cent of the moist weight of the glands. Also the individual animals showed a somewhat wide variation, some having as little as 1.04 mg. per gram of gland, others having from 1.75 mg. to 2.60 mg. and one was found with 2.98 mg. per gram. This great variation in epinephrine content made it very difficult to draw conclusions as to the effect of experimental procedures as one animal might normally carry three times as much of the substance as another.

Another source of difficulty encountered at times was the natural tolerance of some of the dogs for physostigmine. While the vast majority of the animals showed marked symptoms from 0.5-1.5 mg. of the alkaloid per kilogram an occasional animal was encountered which was resistant and which showed little effect from such doses. As was to be expected the adrenals of these animals showed

no marked deviation from the normal in epinephrine content. For example, in the first group of "4 or 5 hour" physostigmine dogs one of the group to which was given 0.78 mg per kg showed practically no sign of poisoning and its glands yielded a normal amount of epinephrine, viz., 1.16 mg per gram. This animal was excluded from the final calculations as such an exceptional reaction would complicate the final figures.

Our experiments with physostigmine were accordingly modified from time to time in so far as the dosage and time of administration of the alkaloid were concerned—these being determined by the severity of the toxic symptoms mani-

TABLE I
EXPERIMENTAL RESULTS

Group I Normal Control Dogs			Average of 11 dogs yielded 1.51 mg epinephrine per gram of fresh gland
Group II "4.5 hour" Physostigmine Dogs			These animals were injected between 8 and 9 A.M. and the glands removed about 2 P.M. on the same day
DOSE OF PHYSOSTIGMINE MG PER KG BODY WEIGHT	WEIGHT OF ADRENALS	EPINEPHRINE PER GRAM OF GLAND	
0.8	1.08	0.66	
1.5	1.27	0.53	
1.8	1.06	0.84	Average 0.73 mg
1.0	0.78	0.21	
2.1	1.23	1.40	
Group III "24 hour" Physostigmine Dogs			Glands were removed the day following the injection of the physostigmine
DOSE OF PHYSOSTIGMINE	WEIGHT OF ADRENALS	EPINEPHRINE	
2.99	1.39	0.43*	
1.78	1.11	0.81	
1.77	0.89	0.75	
2.06	0.73	0.27	
2.00	2.04	0.15*	
2.00	0.85	0.88	Average 0.55 mg
Group IV "48 hour" Physostigmine dogs			Glands removed the second day following the injection of physostigmine
DOSE OF PHYSOSTIGMINE	WEIGHT OF ADRENALS	EPINEPHRINE	
1.74	1.34	0.75	
2.29	1.04	0.44	
1.65	1.21	1.28	
2.00	1.63	2.45	
1.80	1.42	1.41	
4.90	0.9	1.60	
1.90	1.62	1.24	
2.83	1.25	3.20	Average 1.55 mg
Group V "72 hour" Physostigmine dogs			3 days between injection of physostigmine and removal of glands
DOSE OF PHYSOSTIGMINE	WEIGHT OF ADRENALS	EPINEPHRINE	
1.82	1.3	0.85	
1.58	1.08	1.39	
2.19	1.34	0.87	
2.00	1.14	2.46	
2.08	2.01	3.98	Average 1.91 mg

tested by the animals. One dog was injected at 11 A.M. and the glands removed at 2 P.M., making only a three-hour interval and the yield from this animal fell within normal limits, viz., 1.28 mg. per gram of gland. This time then was evidently insufficient to produce any marked change in the gland content as the dose of physostigmine employed (2 mg. per kg. body weight) was ample as shown by numerous subsequent animals. Four more dogs were injected with physostigmine during the early morning hours and in the afternoon. Some four to five hours after the alkaloid had been given the glands were removed and the epinephrine estimated as outlined above. These animals form Group II as given in Table I.

In spite of individual variations the average figures given above yield clear-cut results. Following the subcutaneous administration of an adequate dose of physostigmine the epinephrine content of the adrenals is reduced in four or five hours to about 35 or 40 per cent of the normal. Twenty-four hours later the epinephrine is still further reduced. In certain of the dogs it was as low as from one-fifth to one-tenth of the normal (0.15 mg., 0.27 mg., etc.). The symptoms shown by the dogs with moderately low content were quite striking, viz., marked weakness and depression. If the epinephrine content is still lower the depression and weakness is much more marked, and death upon the table usually follows as is indicated by an asterisk in the twenty-four-hour group table.

The forty-eight-hour group of dogs shows that the glands have largely replenished themselves during the second twenty-four-hour period inasmuch as the average content is practically at the normal level. The seventy-two-hour group shows a considerably higher average figure but this is due to the presence in this group of one dog with an abnormally high content, viz., 3.98 mg. If this animal be eliminated from consideration the remaining four give an average within normal limits (1.39 mg.).

The study of the effect of physostigmine upon the adrenals was extended by administering the alkaloid twice daily over a series of days. This method was found to produce a very marked depletion of the medulla of the glands and associated with this poverty of epinephrine were the characteristic weakness and apathy of the animals. Two of the three dogs died on the table and an examination of the adrenals showed extreme epinephrine depletion. One dog weighing 8.6 kg. received an average of 7.5 mg. physostigmine daily, a total of 45 mg. being given. The dog died and the adrenals assayed 0.27 mg. epinephrine per gram of gland. A second dog weighing 6.8 kg. received an average of 8 mg. daily—a total of 98 mg. being given. The animal showed extreme weakness and died on the table its adrenals yielding 0.45 mg. epinephrine per gram of gland. Such low figures seem to be incompatible with bodily vigor and death usually follows when any extra strain is put upon the animal, even a small dose of urethane being sufficient in several of our dogs to cause a sudden fatal termination. It would seem then to be clearly established that in cases where there has been marked depletion of the adrenal medulla the glands can recover more or less completely in twenty-four hours. It will be remembered that Crowden in his recent paper said that eighteen hours is not sufficient but that three days is ample. From our results it would seem that one day may suffice in some cases.

In addition to the use of physostigmine as a means of causing an increase in the epinephrine content of the blood it has been shown by Stewart and Rogoff⁴ that strychnine also has a similar action even when given in doses which are so small that an increase in motor reflexes is not apparent. The action of this alkaloid in increasing the epinephrine output according to these workers is entirely upon the central nervous system as it is absent when the nerves to the gland are cut. A further interesting point concerned the rate of replacement of the epinephrine which had been poured out. It was found that if the nerves to one gland were severed and strychnine injected, the epinephrine content of both glands was essentially the same in all cases. The explanation given was that the epinephrine which had been poured out from the intact gland had been replaced as quickly as it had been excreted so that no depletion was shown. A similar condition had previously been described by Elliott in 1912. Several other workers have studied the action of strychnine upon the adrenals and all, with one exception, have described an increase in the output of epinephrine and in general the action has been ascribed to an effect upon the central nervous system. Some of these results have been discussed in the paper by Harmon and McFall⁶ whose work forms the exceptional finding referred to above. These investigators, employing the denervated heart of cats as a means of estimating epinephrine output, conclude that there is no evidence that strychnine has any action upon the activity of these glands. These findings are not easy to explain as they are at variance with the results reported by so many other workers. The explanation offered by Harmon and McFall is that under the influence of strychnine the animal may struggle due to increased reflex activity and under such conditions an increase in epinephrine may occur but the increase is due to struggling and not directly to the strychnine. This explanation may possibly hold true for certain animals in which strychnine convulsions or struggling have been encountered but would hardly explain the apparent increase in other dogs and cats which may show increased reflex activity but no convulsive movements. Such animals are reported by Edmunds¹⁰ and also by the same writer in collaboration with Lloyd¹¹. For example, in the latter paper it was shown that epinephrine in small doses produces characteristic changes in the total white blood cell count and in the relative proportions of the different varieties of the white cells. The same curve of changes follows the injection of small doses of strychnine in animals which are deeply anesthetized but if the adrenals are removed before the strychnine is administered the curve is entirely different. This holds true, as reported in the paper, in animals showing no convulsive movements whatsoever, the effect on the blood being due to the epinephrine excreted under the stimulant action of the strychnine.

If this is correct and epinephrine is excreted in larger amounts under the influence of strychnine, it must also be formed more quickly by the gland, inasmuch as several workers have reported no diminution in the epinephrine content of glands removed after strychnine administration. These findings are hardly to be doubted except that in each case the estimations have been made by the colorimetric method which in our hands at least is not so reliable as the biologic method of assay. It therefore seemed desirable to reexamine the question in view

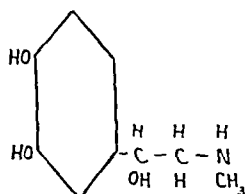
of the findings with physostigmine, and of its bearing upon the general subject of the replacement of this active principle in the gland

Our experiments which were carried out along the same lines as those when physostigmine effects were studied confirm the reported findings that no lessening of epinephrine content is present even when sufficient strychnine is given to produce muscular twitching. The results obtained on one dog may be cited

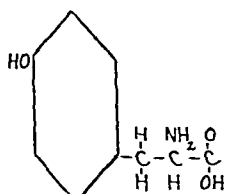
Dog weight, 11 kg. Strychnine sulphate injected subcutaneously as follows: 9:30 A.M. 1 mg., 11:15 A.M. 0.5 mg., 12 noon 0.5 mg., 1 P.M. 0.5 mg. At 1:30 dog showed muscular twitching and some spasticity of legs. 1:45 P.M. dog anesthetized, adrenals removed. Epinephrine content assayed by biologic means and found to be 1.59 mg. per gram of gland. Thus the amount was normal in this animal which showed definite strychnine symptoms.

We tried to produce adrenal exhaustion with strychnine as we had with physostigmine by giving the strychnine over a number of days but with no positive results as the following figures show. Dogs given 0.5 mg. strychnine sulphate subcutaneously twice daily for three days and showing slightly increased reflexes had glands which in one case yielded 1.38 mg. epinephrine per gram of gland and in the other case 2.14 mg. per gram of gland. There was evidently no exhaustion of the glands and whatever epinephrine had been excreted under strychnine stimulation must have been replaced as quickly as it had been poured out. This finding is by no means surprising as it has been repeatedly shown as mentioned above that following numerous stimulations of the splanchnic nerve no lessening in epinephrine content of the glands occurs. There must be a fundamental difference, however, between physostigmine and strychnine in so far as this action is concerned as both increase the outpouring of epinephrine into the blood and both produce this effect by a central action, but while the gland is depleted by the physostigmine no such result follows the use of strychnine or indeed direct stimulation of the splanchnic nerves.

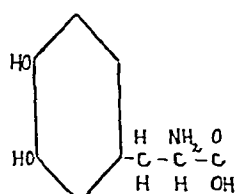
In connection with the synthesis of epinephrine by the animal body there has always been a question as to what is the precursor of the active principle. The two substances which seemed to be the most likely are tyrosine and dihydroxyphenylalanine. These two chemical bodies are quite closely related to epinephrine as can be seen by the following formulae:



Epinephrine



Tyrosine



Dihydroxyphenylalanine

The theory that epinephrine is formed from tyrosine is emphasized by Halle¹² who points out that the four changes in the tyrosine molecule which would be necessary are all perfectly compatible with body metabolism. He further substantiates his views by adding a gram of tyrosine to minced adrenals and incubating the mixture for 7 days. He finds from 14 to 33 per cent more

epinephrine in such a mixture than in one to which no tyrosine has been added. On the other hand Bloch¹³ believes that dihydroxyphenylalanine is a precursor of epinephrine and that in Addison's disease the adrenals are unable to utilize the compound and it therefore is responsible for the pigment formation characteristic of this disease.

In an effort to shed some light upon this question we studied the effect of administering these two compounds to dogs in which the adrenals had been subjected to depletion by physostigmine, as described above. The form of normal recovery curve having been well established, any favorable influence which might be exerted by either of the compounds would show itself probably by a more complete or more rapid recovery than is gained without their use. The experiments were carried out as follows. The dogs were injected with the necessary dose of physostigmine as in the earlier series, and at the end of twenty-four hours when the epinephrine content would be at its lowest level either tyrosine or dihydroxyphenylalanine was administered. In some animals twenty-four hours were allowed following the injection of the suspected precursor before the glands were examined, while in other dogs a second injection was given the next day, so that forty-eight hours were allowed for recovery. The tyrosine solution or suspension caused considerable irritation when it was given subcutaneously so we changed the procedure and gave it intraperitoneally. One-gram doses were stirred for some time in water kept just above body temperature, some NaOH having been added to the mixture. The dihydroxyphenylalanine was prepared in the same way in doses of 200 mg. The results of the combined physostigmine and tyrosine experiments are as follows. It is unnecessary to give all the detailed figures of the thirteen dogs injected with the tyrosine so we will give merely the final assay figures of these animals and the average content in milligrams per gram of gland: 1.31, 1.10, 0.91, 0.83, 0.77, 1.13, 0.51, 0.77, 1.50, 0.66, 2.07, 1.30, 2.25, an average of 1.16 mg. as compared with an average of 1.55 mg. for the dogs which had had no tyrosine. The series of dogs (12) receiving tyrosine on two days yielded results as follows: 1.31, 1.16, 0.84, 0.89, 0.60, 1.81, 0.70, 0.55, 0.62, 0.94, 2.10 mg., an average of 1.04 mg. as compared with 1.91 mg. for the dogs without tyrosine. It is very clear that under the conditions of the experiments tyrosine exerted no favorable influence upon epinephrine replacement which would be expected if it were a precursor of the alkaloid. A similar conclusion was reached by what we may term clinical observation. The dogs given tyrosine were certainly not improved by the drug and in many instances their general condition was worse. A similar study was made with dihydroxyphenylalanine with results which were essentially the same. The glands of three dogs receiving the injections yielded twenty-four hours later epinephrine as follows: 1.50, 2.05, 1.68 mg., an average of 1.74 mg. as compared with the control of 1.55 mg. It is true this figure is higher, but it can hardly be said to be outside the normal limits. An animal given the compound on two days yielded 1.34 mg. of epinephrine as compared with the control figure of 1.91 mg. Dihydroxyphenylalanine therefore does not seem to be beneficial although viewed from other standpoints the negative evidence is not quite so conclusive as it is with tyrosine. For instance we treated dogs with daily injections of dihydroxyphenylalanine for about

two weeks with the following results. One dog weighing 7.7 kg received 2.7 G in divided doses between June 5 and 18 and its glands assayed 1.98 mg per gram of gland. A second dog weighing 10.2 kg received 1.1 G between June 20 and July 21 and its glands yielded 1.31 mg, an average for the two of 1.64 mg which is above the normal average.

Finally as we pointed out earlier there are in all the series of dogs certain of the animals which yield exceptionally high figures, up to three or four times the average value, and such figures with only a few animals in the series profoundly modify the course of the curve. We have accordingly in drawing a graph omitted these exceptional animals from consideration fully realizing that from the statistical point of view such an arbitrary limitation would not be permissible. The justification for such action is seen of course, when a figure of

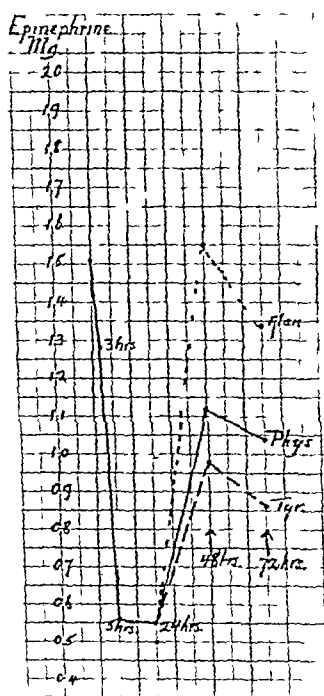


Fig. 1.—Curve of epinephrine content of adrenals of dogs at time periods marked on curves. Solid line shows epinephrine when physostigmine is used alone. Interrupted line (Tyr) when tyrosine is also given as described in the paper and dotted line (Ala) when dihydroxyphenylalanine is given with the physostigmine.

3.98 mg is found in a series the other members of which average about 1 mg. Again a dog with 3.20 mg epinephrine is found in a group averaging about 1.4 mg. Drawing such a curve (Fig. 1) we find that the dihydroxyphenylalanine figures are distinctly above the normal controls in every instance and the findings agree fairly well with the dihydroxyphenylalanine figures for continuous administration. In each instance the seventy-two-hour value is less than the forty-eight-hour figure. Why this should be we cannot suggest but the parallel findings in the curve would seem to offer some justification for the liberty we have taken in dropping the exceptional figures when the curve is constructed. At the same time these are to be found in the tables given.

SUMMARY

The intramuscular injection of physostigmine in dogs is followed by a diminution in epinephrine content of the adrenal glands at the end of five hours to about one third of the normal, and at the end of thirty hours the depletion is even greater. Such animals exhibit marked symptoms of weakness and apathy. The glands gradually replace the active principle, so that at the end of another twenty-four hours the amount in the glands approaches the normal.

If physostigmine administration is continued over a period of two or more days, the glands are depleted to such an extent that they contain only about an eighth of the normal amount. Such animals are very weak and they may die. Studies were also made upon animals with such depleted adrenals to see whether evidence could be secured upon the existence of a possible precursor substance for the epinephrine. For this purpose tyrosine and also dihydroxyphenylalanine were administered, and the adrenals were then examined at varying intervals to see whether the course of the curve as described above was changed. Tyrosine apparently had no effect, but the dihydroxyphenylalanine seemed to exert a favorable influence, but a positive statement could not be made on account of the wide variations between different animals.

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POLIOMYELITIS*

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WITH poliomyelitis again making its appearance in Southern California a review of the 1930 epidemic in this locality is apropos at this time. Particularly is this true in view of the difference in clinical findings and treatment of our cases from those reported elsewhere. The following remarks are based on observations from 350 cases.

The known etiologic facts concerning polio have been reviewed extensively in the literature the past year and only such points as are pertinent to this discussion will be mentioned. The spread by direct contact or "healthy carriers" was exemplified. One of the Los Angeles City Health Department inspectors who speaks Mexican fluently, made a trip through Sonora and Baja California, just preceding the outbreak of the epidemic in this state. He found large numbers of funerals being conducted in every Mexican town and hamlet and was assured that children were dying from the disease "which leaves them paralyzed if they get well". Soon after this the disease manifested itself in Imperial, San Diego and Riverside counties from whence it was traced directly within a few days into Los Angeles County. It is also interesting to note that from here it spread directly north into California as well as to the states east of us along the mainly traveled United States highways.

A normally high immunity is accepted¹ and whether natural or acquired by previous, mild, unrecognized infection, the use in a few instances of pooled nonspecific adult sera gave results comparable to those obtained with convalescent serum. A shortage of the latter made this procedure necessary early in the epidemic.

The invasion symptoms most frequently encountered were those common to most toxemias, particularly those of an acute infectious nature. Frontal headaches, pain and stiffness of the neck and back, constipation with nausea or vomiting, and some degree of fever were the most constant. Little children, among whom headache of any type is a rare complaint, and who usually pay little attention to their bowels frequently of their own accord mentioned both the headache and the constipation. Rarely there was an early diarrhea. The older patients frequently suffered almost unbearable pain in the frontal region "unlike anything previously experienced". Often the degree of prostration was so extreme that it was difficult to obtain a history. The extremely toxic or the bulbar types were lethargic and stuporous, sometimes irrational and usually irritable and hyperesthetic when aroused. A few were unduly alert. Cerebral manifestations such as diplopia, blurring vision, dysarthria, vertigo and incoordination of muscles were common. These early cerebral symptoms frequently presaged a high involvement of the nervous system while those with lower extremity pains

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frequently terminated with lower cord lesions. An extreme sensitiveness to slight stimuli was very noticeable. Deep muscle pains especially in adults was often unduly acute. Sometimes these pains made their appearance long after the fever and other systemic symptoms had subsided (posterior poliomyelitis). Urinary retention was occasionally present early but rarely persisted as the pathology of poliomyelitis does not involve the sympathetic system. Many cases started very insidiously with mild symptoms and a few showed focal symptoms almost as soon as any illness was recognized.

Examination was often entirely negative at this stage, although stiffness of the neck with acute pain along the spine when anteflexed was a common early finding. Whether this be due to stretching of the spinal muscles or to tension on the dura of the cord, it is a suggestive sign of early central nervous system invasion and frequently precedes the positive spinal fluid findings. Hyperactivity of both deep and superficial skeletal reflexes was an early neurologic finding, although the latter were frequently absent when the patient was first seen. Occasionally nystagmus or other evidences of cranial nerve irritation were noted in the systemic stage.

The average case in this series first presented itself at the hospital on the fourth day of illness and at that time every case showed some degree of fever. The maximum was 105° F with an average of 102. Frontal headache was complained of in 70 per cent of cases although many small children and unintelligible foreigners gave no subjective history. Neck rigidity was present in 95 per cent of cases although pain in the neck and lumbar region was mentioned by only 32 per cent of patients. Pain on anteflexion of the spine however, was an almost constant finding, and was almost invariably accompanied by Brudzinski's sign. On admission, 18.3 per cent presented localized pain in one or more extremities. The severity of the headache probably distracted attention in many cases from the milder pains elsewhere. Gastrointestinal symptoms were as follows: constipation 92 per cent, diarrhea 2 per cent, nausea 60 per cent, vomiting 40 per cent, pain in abdomen 3 per cent, difficulty in swallowing 0.86 per cent. Urinary findings included incontinence in 8 cases and retention in only one. Only three cases of the 350 showed convulsions or gave history of same. Fifty and two tenths per cent showed some localized paresis and 11.7 per cent showed definite paralysis on admission.

Following the invasion stage the usual sequence of symptoms followed. The actual number of the abortive type was difficult to estimate because of the uncertainty of the diagnosis in cases not developing central nervous system symptoms. Sixty per cent made complete recoveries before leaving the hospital and thus obviously included some of the 50 per cent who showed localized pareses on admission. A rather unusual observation among the diomedary types was the occasional occurrence of pains and paresis as long as three weeks after the initial preparalytic onset and after an interval of two weeks of freedom from all symptoms. Some of these patients had been sent home with diagnosis other than poliomyelitis. In those cases which made a gradual transition from the systemic stage to that of the central nervous system invasion, the first symptoms were usually pain and weakness of the affected part. Asymmetry of the reflexes was the first objective evidence, and frequently the only deciding factor on which to base a

diagnosis. Peculiarly, however, the abdominal, spinal, or gluteal muscles frequently showed the earliest reflex changes but rarely any residual paralyses. As is usual in poliomyelitis, none of our patients showed residual bladder or bowel paralyses.

Of the various laboratory procedures only the spinal fluid findings were of diagnostic significance. The usual changes were a slight increase in pressure of from 2 to 10 mm. of mercury, slight increase in globulin, increased cell count (mostly lymphocytes), and a characteristic colloidal-benzoin precipitation test. Occasionally the spinal fluid was entirely unchanged, especially in the mild infections and a diagnosis then had to be made on clinical findings alone. Also in the early systemic or preparalytic phase of the disease the spinal fluid findings were frequently negative and misleading and we learned in questionable cases to defer puncture (but not treatment) until some clinical evidence of neurologic involvement, principally changing or asymmetrical reflexes or painful anteflexion of the spine, was present. Quite early in the epidemic a few cases diagnosed "upper respiratory infection," etc., on the strength of negative spinal fluid findings later developed muscular paresis. Thereafter these doubtful cases were given intravenous or intramuscular serum and the diagnosis later confirmed by subsequent puncture or positive clinical findings. This procedure raised the question whether or not convalescent serum, if given to normal individuals might not itself be responsible for spinal fluid changes, but a few patients thus treated who made uneventful recoveries (probably not poliomyelitis) were later tapped and their spinal fluids found to be entirely negative, thus nullifying this contention. In our series 70 per cent of spinal fluids were under increased pressure, and cell counts ranged from 0 to 1083 per cmm., with an average of 87 cells per patient. Differential counts showed lymphocytes predominant in 95 per cent of cases, polymorphonuclears in 3 per cent and an equal distribution of both types in the remaining 2 per cent. In 79 per cent of cases, the colloidal-benzoin test was typical, i. e. negative precipitation in the first five tubes, maximum precipitation in the second five, and decreased or absence in the last five. It was totally negative in 8.6 per cent, showed the tuberculous meningitis type of curve in 8.6 per cent of cases, was "atypical" in 0.8 per cent and questionable or not reported in 3 per cent. *In 12.86 per cent of cases the spinal fluids were negative cytologically but the patients showed characteristic neurologic findings*, while 22 per cent of cases were negative neurologically (abortive type) but showed conclusively positive spinal fluids. Blood counts in our series ranged from 6,000 to 18,000 with an average of 13,000 and 79 per cent polynuclears, obviously not alone characteristic of poliomyelitis. In only a few cases where diagnoses were doubtful were blood cultures or blood chemistry determinations done, and these were all negative or of no positive diagnostic value in favor of poliomyelitis.

The diagnosis of the disease in its early stages is difficult and often impossible. An obvious toxemia, particularly if there is undue prostration, and the cardinal symptoms of frontal headache, fever, stiffness, and pains in the back and in the neck aggravated by anteflexion and some degree of gastrointestinal upset accompanied by constipation is almost diagnostic of the preparalytic phase, particularly in the presence of an epidemic. If to the above is added evi-

dence of central nervous system involvement, especially asymmetrical or changing reflexes, unilateral weakness, pains, or paresthesiae, the suspicion is strengthened. If the spinal fluid then shows an increased pressure, globulin, or increased cell count with a preponderance of lymphocytes, the diagnosis is almost certain. Polomyelitis, if seen in the preparalytic stage, must be differentiated from all acute infectious diseases, particularly acute upper respiratory or gastrointestinal toxemias. Of the former, the epidemic (streptococcus) sore throats were the most common in our series, although several cases of ordinary coryza and sinusitis, and a few cases of influenza (questionably abortive polomyelitis), otitis media, bronchitis, and pneumonia were seen. Two cases of pyelitis, in which the local symptoms were overshadowed by the systemic, were admitted to the hospital as suspects, also several cases of hysteria. Encephalitis, although rare, was seen in a few instances at this time, although here again the question of etiology (bulbar involvement) was present. Meningitis, particularly the tuberculous type, was frequently seen and, rarely, confused for a short time with the bulbar type of the disease. A purely clinical differentiation between tuberculous meningitis, encephalitis and bulbar polomyelitis is frequently most difficult if not impossible, and in these diseases the colloidal-benzoin test has been of great help. Osteomyelitis was seen for differentiation in several instances, and also an occasional case of multiple neuritis (especially alcoholic), acute rheumatic fever, central nervous system syphilis, brain abscess, and cerebral accident. One case each of meningococcus septicemia, acute endocarditis with embolic phenomena and acute reticuloendotheliosis were seen during this epidemic. In infants, birth injuries, scurvy, rickets, and congenital muscular weakness were all forwarded to the service for differentiation from polomyelitis. One case of polomyelitis was complicated by the presence of scurvy. The diagnosis in some few cases was, and always will remain a mystery, but in a majority of instances the ultimate findings were conclusive. In time of epidemic, a communicable disease hospital always receives a large number of cases for differentiation from those of the prevailing illness.

There were many toxemias. Some of these patients recovered spontaneously and others later showed localizing symptoms. Of the spontaneous recoveries only those showing cytologic changes in the spinal fluid were considered as abortive polomyelitis. Those with paresis were obviously all polomyelitis, but, as above stated, *and contrary to current medical opinion*, 12.86 per cent showed no cytologic changes in the spinal fluid. Of those patients with obvious sources of their toxemia (particularly the septic sore throats), and who developed no neurologic findings, only those were considered as positive whose spinal fluids showed cellular changes. All of these fluids also showed typical colloidal-benzoin curves. This test we believe to be of greatest relative value. Toxemias are common in many diseases and the "meningismus" often seen is as much a part of the systemic manifestations of the disease as the headaches or the skeletal pains. That changes should occur in the spinal fluid in such cases is not inconceivable, and in a few instances we did spinal punctures on patients with various other acute infections for the purpose of evaluating the colloidal-benzoin test. All showed some degree of precipitation but no cellular changes, and these were classified as extreme toxemias involving the central nervous system and not as abortive polomyelitis.

myelitis Irrespective of etiology poliomyelitis is an anatomic and pathologic entity, and localizing symptoms formerly discussed when clearcut, justify us in so diagnosing the case

The hospital treatment of poliomyelitis during this epidemic was largely with pooled convalescent human immune serum plus the usual rest immobilization of affected extremities dietetic and eliminative measures Occasionally methenamine was rather empirically used because of its supposed diffusion into the spinal canal, and calcium for its muscle tonic effect was likewise given with about the same degree of expectancy Hypertonic saline intravenously was used in a few cases when serum was not available Previous experience with Rosenau's serum has caused us to abandon its use and the one patient in this series who received it before admission to the hospital developed the all too common allergic reaction, a terrific urticaria The amount of convalescent serum given averaged 15 cc per patient when given intrathecally and 30 cc when given intramuscularly or intravenously The choice of method was determined by the stage of the disease the severity of onset the certainty of diagnosis, and evidence of central nervous system involvement It has been definitely established that dyes given intramuscularly and intravenously do not appear in the spinal fluid for at least twenty-four hours, and we feel very strongly that if the toxin has reached the central nervous system as evidenced by neurologic and spinal fluid findings that the antidote should be given intrathecally as well as intravenously in order to neutralize the toxin in its most dangerous site without unnecessary delay Similarly we feel that when the disease is still a toxemia without central nervous system phenomena, that the intravenous or intramuscular routes may be the most logical Having had experience with intracisternal therapy in this hospital, where over 1 000 punctures have been done in the past year for the treatment of various conditions, we have generally preferred this approach when giving serum intrathecally The criticism of intrathecal medication because of irritant preservatives in the serum seems illogical in view of the splendid results obtained with antimeningococcic, antitetanic, and other sera given this way all of which contain preservatives and which also have the added disadvantage of being horse sera to which a patient may show an allergy Reactions to medication were shown in only five of our cases and these were very mild and transient

While it is difficult to estimate the value of any therapeutic measure in this disease, which is too serious to permit of untreated controls and which normally includes such a high percentage of spontaneous recoveries, nevertheless our results seem to warrant our method of treatment The intracisternal injection of serum seemed to be the turning point in the febrile stage of the disease in 82 per cent of the cases and the subjective symptoms likewise seemed to rapidly abate following its administration We were able in one instance, to definitely arrest a case of the ascending or Landry's type with intracisternal medication, a precedent in this hospital The number of complete recoveries (60.9 per cent), the 25.66 per cent of cases with very mild residual paresis all of whom will probably recover fully, the 10.28 per cent of residual paralyses most of whom are improving, and the low death rate of 3.16 per cent point to a mild type of the disease early recognition, or efficient treatment The death rate for Los Angeles

County during the same epidemic, as given by the Health Department, was over 7 per cent. In as much as this figure is for the same community and the same epidemic but includes patients treated outside the hospital, we feel that our treatment must have been a factor in the low death rate within the institution. Judging from data on previous epidemics in this community, there is no reason to believe that the disease was milder in 1930 or in this locality than elsewhere. Our results, as contrasted with methods outlined in *California and Western Medicine* for October, 1930, have been accomplished with very much smaller doses of serum, not that we may not have desired in many instances to have given more, but because the inadequate supply required conservation, and from the results, the dosages used seemed ample. We feel that the relatively low morbidity rate here was largely due to the cooperative efforts of the city and county health authorities and the private physicians resulting in early recognition of cases and their rational treatment.

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CARBON MONOXIDE ACUTE AND CHRONIC POISONING AND EXPERIMENTAL STUDIES*

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CARBON MONOXIDE AFFINITY FOR HEMOGLOBIN, ACUTE AND CHRONIC POISONING, EXPERIMENTAL STUDIES OF EFFECT OF AUTOMOBILE EXHAUST GAS ON GUINEA PIGS

HEMOGLOBIN is very widely distributed throughout the entire animal kingdom, being found in the blood corpuscles of mammalia, birds, reptiles, amphibia and fishes, it is also found in the blood corpuscles of many of the invertebrates. The composition of its molecule varies somewhat in the different animals, so that, strictly speaking there are probably a number of different kinds of hemoglobin, all however, closely related in chemical and physiologic properties.

Hemoglobin has the property of uniting with oxygen in certain definite proportions, forming a true chemical compound, known as oxyhemoglobin. Each molecule of hemoglobin unites with a molecule of oxygen. Oxyhemoglobin is not a very firm compound. If placed in an atmosphere containing no oxygen it is dissociated, giving off free oxygen and leaving behind hemoglobin or so-called reduced hemoglobin. This power of combining with oxygen to form a loose chemical compound, which in turn can be dissociated easily when the oxygen pressure is lowered, makes possible the function of hemoglobin in the blood as the carrier of oxygen from the lungs to the tissues.

There are a number of pigmentary bodies which are formed directly from hemoglobin by decomposition or chemical reactions of various kinds. The best known and most important are methemoglobin, nitric oxide hemoglobin and Carbon Monoxide hemoglobin.

METHEMOGLOBIN

This pigment is closely related to oxyhemoglobin, since it contains the same amount of oxygen and is isomeric with it. The oxygen is however, not in loose combination and cannot therefore be utilized by the system.

NITRIC OXIDE HEMOGLOBIN

Nitric oxide forms a firm compound with hemoglobin rapidly oxidizing it to methemoglobin.

CARBON MONOXIDE HEMOGLOBIN

This pigment is a molecular combination of one molecule of carbon monoxide with one molecule of hemoglobin. It forms a stronger combination than oxygen and hemoglobin, carbon monoxide having an affinity for hemoglobin approximately three hundred times that of oxygen. Since carbon monoxide readily

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combines with both oxyhemoglobin as well as with reduced hemoglobin, the tissues suffer for want of oxygen. Carbon monoxide hemoglobin imparts to the blood a bright, cherry red color both in the venous and arterial circulations and can be readily recognized by a number of standard tests.

CARBON MONOXIDE

Carbon monoxide is a colorless, tasteless, odorless gas. Its most common sources are coal stoves, grates, salamanders, domestic and industrial furnaces, gas engines, coal, natural, and artificial gases. It is formed whenever incomplete combustion of carbon occurs or, as a matter of fact, anywhere where combustion of the carbon takes place slowly with an insufficient quantity of oxygen. The first stage of combustion produces the poisonous gas CO. A second stage then produces comparatively harmless gas, CO₂. In the first stage each carbon atom unites with one atom of oxygen, in the second stage with two atoms of oxygen.

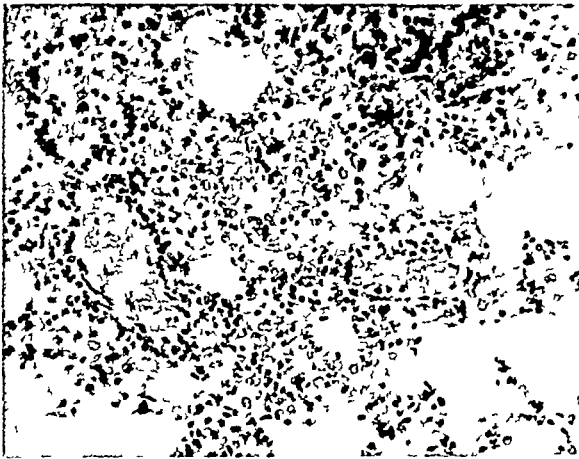


Fig 1—Guinea pig lung ($\times 175$). Shows the hyperemia and the localized areas of consolidation. These changes are not unlike those found in any section of guinea pig lung and are not specific for carbon monoxide poisoning.

In a coal fire the process may be different, the combustion taking place in the lower layers of red hot coals may be complete but the CO₂ passing upward through the less heated layers may give up some of its oxygen or take up more carbon, forming carbon monoxide.

In burning buildings carbon monoxide may be produced in large amounts and is the chief constituent of the smoke which overcomes the firemen. In fires and explosions in mines it is the carbon monoxide that often causes more deaths than the explosion or fire itself.

Tobacco smoke from cigarettes contains considerable carbon monoxide. McNally¹ found that the carbon monoxide from inhaled smoke was from 0.014 to 0.026 per cent of the tobacco and paper consumed, from cigars it was from 0.027 to 0.015 per cent and from pipe tobacco 0.027 per cent. This averages about 80 c.c. of carbon monoxide to each gram of tobacco burned.

The proportion of carbon monoxide differs greatly in domestic and industrial gases. The commercial gas served to the consumer, which is a mixture of gases

from wood coal and naphtha contains about 11.25 per cent of carbon monoxide. An atmosphere containing 0.2 per cent is capable of destroying life. Haldane²

AUTOMOBILE EXHAUST GAS

The internal combustion engine where a mixture of air and gasoline vapor is caused to burn produces carbon monoxide in the exhaust varying anywhere from 3 to 10 per cent. The richer the mixture the more of the CO gas produced. As a rough figure it has been estimated that one cubic foot of CO gas is produced per minute for each 20 horsepower of the motor. From this one can estimate how quickly a small garage can be polluted to a concentration sufficient to overcome a person in the enclosure and to produce death in a short time.

Analysis of street air has shown carbon monoxide in varying amounts but never in sufficient amount to be distinctly poisonous. The gas escaping from the



Fig. 2—Guinea pig kidney ($\times 100$). Acute CO death shows active hyperemia and a small hemorrhagic area.

automobile exhaust is rapidly diluted by the surrounding air so that one standing behind a car not in motion but with the engine running, would be in an atmosphere of about 4 parts to 10,000. Carbon monoxide concentration in the air must exceed 15 parts per 10,000 to produce possible serious results.

The atmospheric concentration times the time of exposure determines the amount of absorption. Police officers on traffic duty in the large cities have shown as high a concentration as 30 per cent saturation of the hemoglobin with carbon monoxide. The New York Division of Industrial Hygiene in 1923 in a survey of 157 garages, repair shops and service stations found carbon monoxide positive in 69.5 per cent of the workers and carbon monoxide was present in the air in appreciable amounts in 77.5 per cent of the places examined.

Henderson³ in a study of the exhaust system for the New York, New Jersey vehicular tunnel made the following observations:

1. When the time of exposure in hours times the concentration of the CO in parts per 10,000 equals 3, there is no appreciable effect.
2. When the result is 6 there is just a perceptible effect.

3 When the result is 9 there will be headache and nausea

4 When the result is 15 or more the conditions are dangerous to life

ACTION

Carbon monoxide may be freely respired, causing no irritation of the air passages, but the moment it comes in contact with the blood by diffusion, it unites with the hemoglobin forming carbon monoxide hemoglobin

Hill and Baicroft⁴ have determined that CO combines more readily with unsaturated oxyhemoglobin than with hemoglobin, in other words, hemoglobin will take up more CO at a given tension if a little oxygen is present than if oxygen is completely absent

Nieloux⁵ has shown that the red blood cells even when saturated with carbon monoxide are not devitalized but are ready to resume functioning when supplied

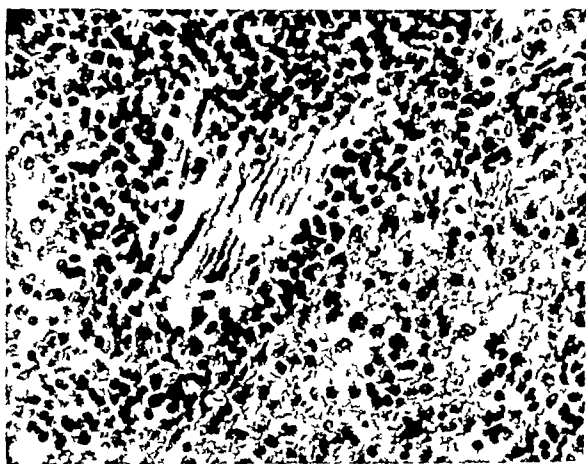


Fig 3—Guinea pig spleen (x175) Acute CO death pulp space lower right hand corner filled with blood active hyperemia

with oxygen Blood containing CO hemoglobin may be deprived of the gas by submitting it to diminished pressure or by passing air or oxygen through it for a considerable length of time

The development of poisoning from carbon monoxide depends entirely upon the concentration of the CO in the air and the time of exposure The higher the concentration of CO in the inspired air, the greater the saturation of the blood with corresponding decrease in the time of exposure For instance 0.02 to 0.03 per cent CO in the inspired air will only cause a 23 to 30 per cent saturation of the blood in five to six hours whereas 0.5 to 1.0 per cent CO will cause a 73 to 76 per cent saturation of the blood in two to five minutes

SYMPTOMS AND EFFECT UPON THE BODY

The symptoms depend upon whether the poisoning is acute or chronic In the acute cases the symptoms depend upon the concentration of the CO in the inspired air and progress according to the degree of concentration present

As anoxemia progresses the blood pressure is at first increased as a result of reflex stimulation of the vasomotor center, later the pressure is decreased due

to a benumbing of this center and a dilatation of the blood vessels. Apoplexy may occur in the first stage. The pulse becomes slower as the blood pressure rises, the heart beats violently, subsequently the pulse becomes frequent but small. The breathing is deep and difficult as a result of deficient oxygenation and the diminished production of CO_2 . Headache, throbbing of the temples, tinnitus, faintness, dizziness, vertigo and even vomiting may occur. The face becomes flushed, more or less extensive patches of bright red color make their appearance on the surface of the body. The muscular system is quite early affected, special muscle groups may become paralyzed, there may be tonic or clonic spasms. Involuntary urination and defecation may occur. The body temperature is lowered, unconsciousness may occur early which is especially true when ethane gas is present with the CO. Death occurs when the respiratory center is paralyzed. This usually occurs when the blood becomes saturated to about 70 to 80 per cent.

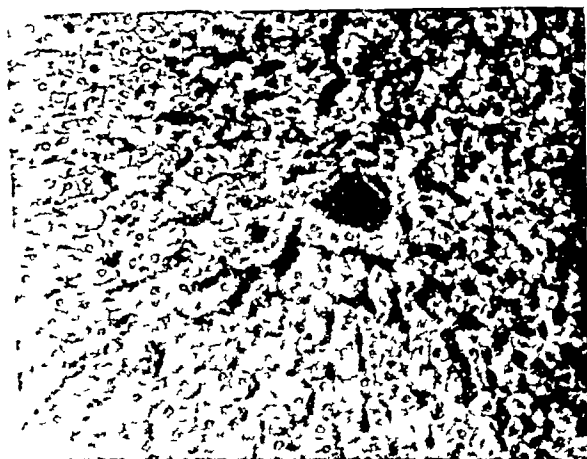


Fig 4—Guinea pig liver ($\times 175$). Photograph taken using blue filter to bring out portal capillaries shows active hyperemia around central vein.

If removed in time recovery sometimes takes place rapidly, but more usually symptoms persist for some time. Muscle paralysis and even degeneration may continue. Sensations to pain may remain absent for a long time.

Various opinions may be found in the literature as to the action of CO upon the nervous system and other body tissues. The inhalation of oxygen with 20 per cent CO has been reported to have caused cramps and total paralysis within the first minute of inhalation which could certainly not have occurred because of the anoxemia itself. Kobert,⁶ Geppert,⁷ and others strongly incline to the belief in its action upon the nervous system, both on the peripheral nerves and on the ganglion cells of the brain, and they extend the poisonous action of the gas to the production of a degeneration of the muscles and glands.

Haggard⁸ has demonstrated that CO has no direct toxic action upon the nervous system. Haldane² and his coworkers have generally concluded that CO unites only with hemoglobin and that it owes its toxicity solely to the fact that it thus interferes with the oxygen carrying power of this compound. It is possible, however, that it unites with other oxygen receptors as well as those of the

hemoglobin and it may thus act directly on cells. It is found to be somewhat more toxic, especially toward the basal ganglia of the brain, than equivalent asphyxia for mammals, which would seem to bear out this view.

Other sequelae that have been mentioned are primary gangrene, blisters, decubitis, persistent distention of the capillaries, pneumonia, and deep seated disturbances of degeneration of all the organs, especially of the vascular walls and ganglion cells.

CHRONIC CARBON MONOXIDE POISONING

Chronic carbon monoxide poisoning occurs as a result of breathing small quantities of carbon monoxide over long periods of time. Digestive disturbances, diminished vigor, coated tongue, loss of memory, more or less muscular weakness are all reported as occurring. Some workers have reported anemia, simulating

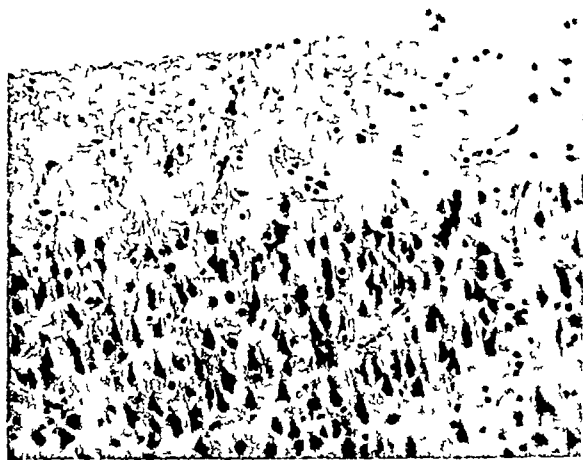


Fig. 5—Guinea pig brain cortex ($\times 175$) Shows considerable edema pyramidal zone normal

the pernicious type, others including Davis (quoted by Forbes),⁹ Hames¹⁰ have reported cases of polycythemia, sometimes the count running as high as six to nine million with a hemoglobin above normal. It is claimed that the increase in the red cells is a protective effort on the part of the system.

Gruber¹¹ has shown that CO is not a cumulative poison, when inhaled in very small amounts it rapidly disappears from the blood. Therefore the blood examination may not be an aid to diagnosis in chronic cases.

TREATMENT

Much could be said in regard to the prevention of CO poisoning. The incidence of this form of poisoning far exceeds that of all other forms of poisoning combined and yet it is surprising how little action has been taken to prevent its occurrence. By this I mean, legislative action.

In some states regulations have been passed and rigid inspections of the sources of origin of the gas are carried out. Education of the public in this matter seems to be considerably neglected, and it is only occasionally that it is

called to one's attention by reading of certain deaths having occurred from this source

During a three-year period, 1918, 1919 and 1920, there occurred in New York City and Chicago alone 2916 deaths from poisoning such as wood alcohol, bichloride of mercury, carbolic acid, potassium cyanide, strychnine, narcotics carbon monoxide, etc. Out of these 2916 deaths 2298 were caused by carbon monoxide. In these same cities there were 3167 deaths from poisoning during 1928 and 1929, and 2628 were due to carbon monoxide poisoning.

Carbon monoxide asphyxia if untreated, may continue in force for a long time even after the patient is removed from the poisonous atmosphere. Early inhalation treatment to assist in the elimination of this gas from the blood is essential. Oxygen 93 per cent with carbon dioxide 7 per cent, seems to be the inhalation of choice. The carbon dioxide acts as a stimulant to the respiratory

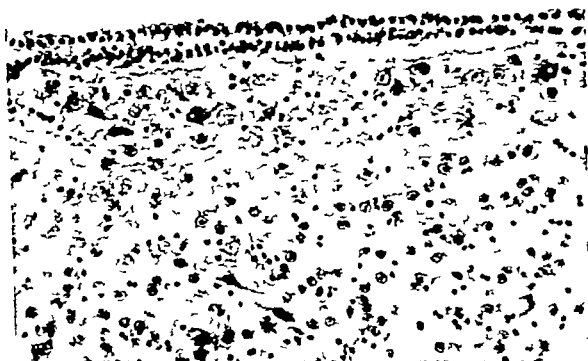


Fig 6—Guinea pig brain floor of ventricle basal ganglion ($\times 175$) Some edema ganglion cells normal no hemorrhage

centers. If respiration has stopped, artificial respiration should be given by the prone pressure method and continued until it is seen to be useless or success is attained. If the carbon dioxide is not available, oxygen alone or even fresh air is of value. The victim should not be moved in severe cases except to remove him to a fresh air atmosphere. Warmth should be applied and it is important that the victim be placed upon warm rugs or garments and not on the cold floor or ground.

Hypodermic medication is useless. When the patient can be moved he should be taken to his home or to a hospital for observation and care. Blood transfusion is also valueless, probably due to the fact that CO combined with tissue receptors becomes released and enters the infused corpuscles converting the oxyhemoglobin to carbon monoxide hemoglobin. Until the carbon monoxide is largely eliminated from the body cells, this process would occur.

Until the patient is fully restored to normal he should be kept absolutely quiet and not even allowed to sit up. The length of time necessary for elimination of the gas varies of course, with the amount absorbed and the treatment received.

There seems to be quite a variance of opinion as to the rapidity of its elimination, some claim that this change may take place in a couple of hours, others state in from four to six hours and others that the gas is eliminated quite slowly. It is unquestionably eliminated more rapidly if oxygen or oxygen and carbon dioxide are administered but it is very questionable whether this gas is eliminated so rapidly by medium of the air alone. Our experiments have convinced us thoroughly that this is the case.

Out of 43 consecutive cases received at the Cook County Hospital at Chicago, 34 per cent were examined within one half hour after the patient was removed from the source of exposure, CO was found in all. In 30 per cent the exact time was not known but was greater than one half hour, 27 per cent being positive and 3 per cent were negative. In 14 per cent of the cases twenty to forty minutes had elapsed and CO was present in 12 per cent and negative in 2

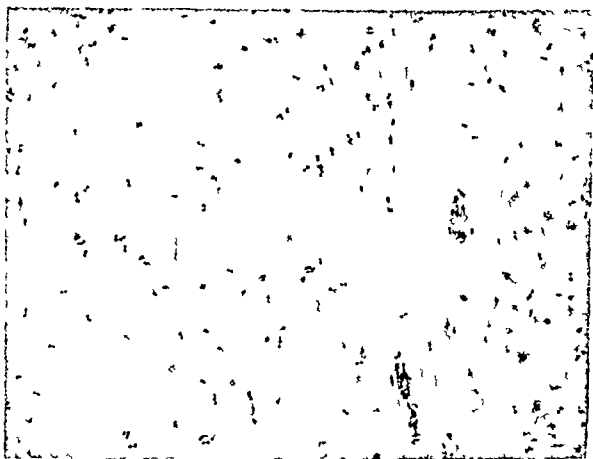


Fig. 7—Guinea pig brain, medulla (x100) Showing ganglion cell areas normal

per cent. Of the 10 per cent examined after three hours all were positive, in another 10 per cent five hours had elapsed, all were positive, and in 2 per cent twelve hours had passed and all gave positive tests. The negative cases found in this series may not have been exposed to quantities of the gas and may have been unconscious from other substances such as ethane which occurs in illuminating gas.

It has been claimed that CO may be in part changed by oxidation into CO₂ within the body. Gruber¹¹ and others claim that this oxidation does not take place but that the CO is voided, quantitatively, unchanged. Whatever process of elimination occurs, it is certain that it is not rapid. The fact that this gas has an affinity for hemoglobin approximately 200 to 300 times that of oxygen would support this claim. On account of the variance of opinions a series of experiments were performed to make a study of this question and to observe the pathologic changes that occurred from inhalation up to varying percentages of blood saturation. A series of guinea pigs were used and a special box, air-tight with full glass top and measuring 4 by 3 by 2 feet, with an inlet for the gas and

a corresponding outlet for the air, and with a door for handling the animals, was designed and used obtaining the CO from the exhaust of an automobile by a connection of rubber tubing. The CO was found to be 8 per cent with the motor running easily. This remained quite constant.

TABLE I
EXPERIMENTS*
(Guinea Pigs)

NO	CO CONCENTRATION	FIRST SYMPTOM	COLLAPSE AFTER	REMOVED AFTER	DEATH AFTER	CO% IN BLOOD	NO OF DAYS CO FOUND IN BLOOD
1	gas started	1 min	3 min.		10 min	70% plus	
2	full sat	at once	40 sec		5½ min	70% plus	
3	full sat	50 sec	2 min		5¾ min	70% plus	
4	full sat	30 sec		2 min	3½ min later	70% plus	
5	full sat	50 sec	1½ min	1½ min	Recovered	60%	31 days
6	full sat	30 sec	1½ min	1½ min	Recovered	55%	25 days
7	full sat	30 sec		1 min	Recovered	40%	10 days
8	½ sat	1 min, 40 sec	5 min		14 min	70% plus	
9	½ sat	1½ min	7 min		24 min	70% plus	
10	½ sat	1½ min		4 min	Recovered	60%	28 days
11	½ sat	1 min, 40 sec		3 min	Recovered	55%	20 days
12	½ sat	1 min, 40 sec		2 min	Recovered	40%	15 days
13	¼ sat	2 min, 30 sec		4 min	Recovered	50%	8 days
14	¼ sat	2 min		3 min	Recovered	30%	5 days
15	¼ sat	no symptom		2 min	Recovered	25%	3 days

*The animals were placed in a special gas chamber connected by tube to the exhaust of an automobile and were observed through a glass top. Special sliding doors were arranged so that they could be removed at any time without disturbing the CO concentration.

The pigs were exposed to atmosphere fully saturated, 50 per cent and 25 per cent saturated. Table I is a summary of this series of experiments.

EXPERIMENTAL OBSERVATIONS

From these experiments it was seen that the pigs that died all showed at least 70 per cent saturation of CO in the blood. In atmospheres not fully saturated where the animal is allowed to breathe the CO somewhat longer, the blood concentration is high when the animal is removed and lives. It is also evident that CO does not rapidly disappear from the blood but is slowly eliminated.

Another series of experiments were performed upon dead animals to determine the ability of the hemoglobin to absorb CO after death.

In 4 guinea pigs killed by etherization, laked blood saturated with CO from illuminating gas, was injected into the muscles, also beneath the skin and into the peritoneal and thoracic cavities. Twenty-four hours later the animals were autopsied and all showed similar appearances to those that had died from inhalation of this gas. This same experiment was performed on two pigs killed by etherization and then injected with embalming fluid. Twenty-four hours later laked blood with CO was injected and the results were practically the same.

Two guinea pigs killed by etherization and embalmed for twenty-four hours were placed in an atmosphere of illuminating gas. These pigs had been previously autopsied and the thoracic and abdominal cavities were open. After

eight hours the tissues and all the blood showed marked evidence of saturation with CO

Two other pigs were killed by etherization and embalmed for twenty-four hours and not autopsied but, with the skin intact, were placed in an atmosphere of illuminating gas, contents of the jar being changed by allowing the gas to enter once an hour for eight hours. The pigs were then kept in the sealed jar overnight. Upon examination of these animals they presented the typical appearances of all the other animals that had died by inhalation of the gas.

These points are of considerable importance from a medicolegal standpoint and were suggested by the experiments of Strassmann and Schultz¹³ who demonstrated that CO may penetrate by diffusion all parts of a cadaver with sufficiently long exposure to an containing this gas. It also brings out the fact that if a body has been buried in a region where gases containing carbon monoxide

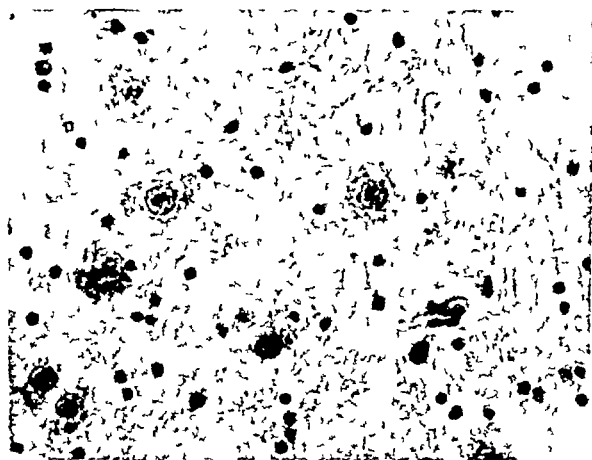


Fig 8—Guinea pig brain medulla ganglion cell area (x375). Same as encircled area in Fig 9. Normal ganglion cells.

are present in the earth, this gas may penetrate the body and be present in the blood in sufficient quantities to lead to an erroneous conclusion that death had been caused by carbon monoxide poisoning. It is also evident that a body may be so tampered with before or after autopsy that an erroneous conclusion may be formed and death attributed to carbon monoxide poisoning.

POSTMORTEM APPEARANCES

The body may present little evidence as to the cause of death, but generally well marked cherry red blotches will be seen on the dependent parts of the trunk, neck and thighs. The eyes are usually closed, the countenance usually composed.

Upon opening the body one is immediately struck by the unusual bright cherry red color of the muscles and of the venous as well as the arterial blood. The blood is usually fluid and is present in the arteries, veins and all of the tissues. The brightness of the blood may be masked by carbon dioxide when it has also been present in the inspired air. When the action of the gas has not been so concentrated and the exposure longer, small hemorrhages may be present in some

of the tissues together with pulmonary edema and a bright red froth in the air passages. The gastric and intestinal mucosa may also show small punctate hemorrhages. The kidneys and liver show few if any changes. Glycosuria has been present in about 20 per cent of the cases that have been examined.

When life has been prolonged, the skin may show blebs, herpes and even gangrene. Some of the muscles may show degeneration. If the patient has lived

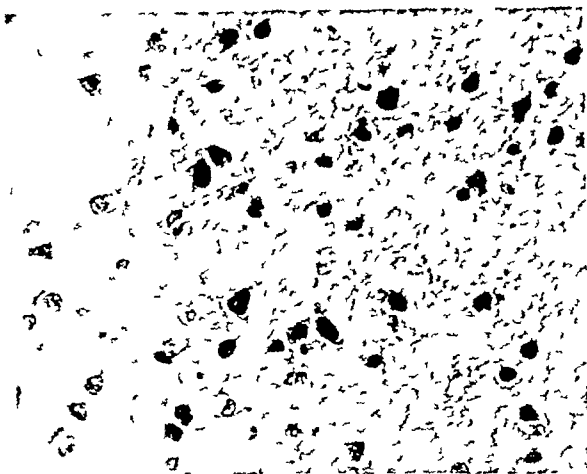


Fig. 9

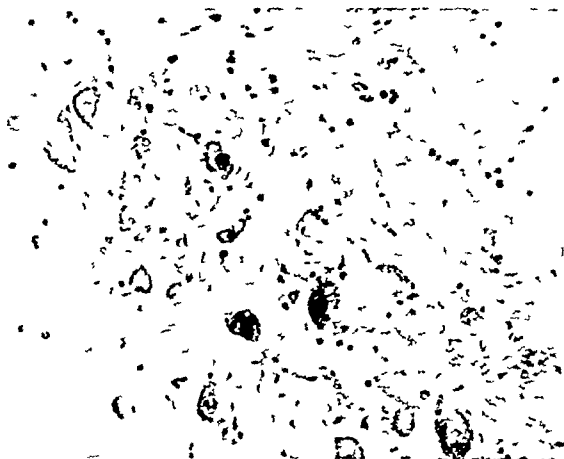


Fig. 10

Figs. 9 and 10 —Areas showing ganglion cells from human brain of acute CO death, normal

for a number of hours only a careful examination of the blood may reveal the presence of the gas. It must also be remembered that the blood is bright red in color when death is due to cyanide poisoning, but then the color is not so permanent and changes to blue upon exposure to the air. Carbon monoxide can be readily recognized by a number of standard tests, principally the sodium hydroxide the potassium ferrioxanide the tramin tests and spectroscopic ex-

amination Another point of interest from a medicolegal as well as diagnostic standpoint is the fact that formaldehyde and embalming fluid do not interfere with the determination of CO in the blood, Tollens¹⁴ even recommends adding some formaldehyde to the blood solutions when making spectroscopic examinations

POSTMORTEM PATHOLOGY MICROSCOPIC

In order to determine the effect of carbon monoxide on the body tissues, sections were made from all the pigs that died during the experiment and from several that lived for varying lengths of time following exposure The parts sectioned included cerebral cortex, basal ganglia, medulla, lungs, heart, liver, spleen, kidneys, suprarenal glands and voluntary muscle

The tissues from the animals that died during the experiment or immediately afterwards presented the following findings

Cerebral cortex in the majority the findings indicated edema, some congestion of the pial vessels and occasionally minute hemorrhages

Basal ganglia moderate edema, no apparent congestion of vessels, no hemorrhages were present

Medulla no variation from the normal with the possible exception of some edema No hemorrhages and no degenerated ganglion cells were seen

Lungs edema, congestion of capillary vessels, areas of minute hemorrhages, with some filling of peribronchial air cells and a moderate amount of exudate especially in the smaller bronchial tubes

Heart muscle showed no variation from the normal

Liver, spleen, kidneys and suprarenal glands all of these tissues appeared normal with the exception of occasional minute hemorrhages and congestion of capillary vessels The most marked changes were in the kidneys where minute hemorrhages were more frequent in the cortical zone

Voluntary muscle none of the sections showed any evidence of hemorrhage or muscle cell degeneration, all sections appeared normal

The tissues from the animals that lived after varying lengths of exposure showed no variation from the normal with the exception of the lungs Evidence of emphysema, peribronchial consolidation and bronchial exudation were present in all The brain tissues and ganglion cells, heart muscle and voluntary muscle showed no evidence of degeneration All the organs sectioned showed no changes other than those usually found in pigs sectioned after death by etherization

These microscopic findings hardly substantiate the claims presented in other reports

SUMMARY

We have presented a study of carbon monoxide from a standpoint of the method of its production, its affinity for hemoglobin, its action upon being inspired, the percentages of blood saturation in varying lengths of time according to the concentration in the respired air, the symptoms and effect upon the body, possible methods of its elimination from the body, the production of chronic carbon monoxide poisoning, methods of treatment, the postmortem macroscopic appearances of the body following carbon monoxide deaths, some important

medicolegal facts, a series of experiments on guinea pigs to ascertain its persistence in the body following exposure and the histopathology of various tissues in animals dying, directly during exposure, and those killed and autopsied at varying periods later

CONCLUSIONS

We admit that this is but a preliminary step in any study of this interesting subject but it does seem from our findings that CO is not rapidly eliminated from the body unless other measures than simple respiration of air are used

It appears that in acute cases death is caused entirely by anoxemia and respiratory failure

The body after death is capable, even after embalming, of absorbing sufficient carbon monoxide, when concentrated and with sufficient time of exposure, to produce all of the macroscopic appearances and positive chemical findings of death due to this gas

It is also possible to produce similar findings by injecting the body with either laked or whole blood, saturated with CO. These possibilities are of grave medicolegal importance

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ENDOGENOUS URIC ACID AND HEMATOPOIESIS*

III URIC ACID OUTPUTS AND RETICULOCYTE COUNTS AS AFFECTED BY GLYCINE, CAFFEINE, UREA, BILIRUBIN, ATOPHAN, AND XyLOSE

By JOSEPH KRAJKA, JR AUGUSTA, GA

MARKED increases in uric acid outputs have been demonstrated by the author (Krajka, 1929, 1930), following regeneration of the red blood cells after severe hemorrhage and after hemolysis. The reticulocytes, or young red cells, present in the peripheral circulation have been numerically correlated with the uric acid outputs. These results support the hypothesis that relates endogenous uric acid to the nucleoprotein derived from the destruction of the extruded nuclei of the normoblasts at the maturation of the erythrocytes.

In a search for the factor responsible for the maintenance of the balance between red cell production and red cell destruction, a check has been made on the various substances known to increase uric acid elimination, such as glycine, caffeine, atophan, and several simple diuretics, together with bilirubin and xylose. The last two are considered because of their relation to red cell destruction in the one instance and nuclear destruction in the other.

EXPERIMENTAL

The majority of the tests were carried out on a pure-bred Dalmatian coach dog. The complete data include daily observations on erythrocyte counts, reticulocyte counts and uric acid outputs for 239 consecutive days. The more significant results were checked on a series of rabbits and one test was carried out on a human being. All drugs were administered by mouth except in cases indicated.

The methods of testing are essentially those used by other investigators, that is, by establishing a base level as a control before administration of the drug. The data are presented in table form.

Glycine—Since Lewis, Dunn, and Doisy (1918), Christman and Mosier (1929), have tested the action of glycine on uric acid outputs, two tests were carried out on our dog. Three grams of glycine (Eastman Kodak Co.) were fed February 17. The uric acid output for the subsequent twenty-four hours was 727 mg. as compared to 324 mg., average for the four previous days. A second dose of 6 grams of glycine on February 18, however, was followed by a uric acid output of only 400 mg. The rise was not consistent for the second dose. The results are best explained as due to the increased elimination since the first dose brought out a total urine volume of 640 c.c., while the second produced only 425 c.c. A similar relationship may be seen in the two investigations cited above particularly in the three experiments of Christman and Mosier.

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In our test the reticulocytes rose from 32,500 per c mm to 52,110 on the subsequent day and dropped again to 6,020, 11,640, 22,520. There was no proportional rise on the third day as would have been expected had a real marrow stimulation taken place. On our hypothesis our data favors the view that glycine increases uric acid outputs by elimination only. The rise and fall in reticulocyte count is explained on the basis of flushing the current crop prematurely, as will be discussed more at length under the tests with caffeine.

The data are given in Table I.

Urea—Under the hypothesis of a specific dynamic action of amino acids, Lewis Dunn and Doisy have tested urea. The dosage which they used had only a very slight diuretic effect and showed no increase in uric acid output. Our initial dose of 20 grams was also evidently subminimal, since no diuresis was obtained and no increase in uric acid was noted over the prevalent level. However, the second dose of 75 grams brought out 1060 mg. on a volume of 1350 cc. at a

TABLE I
EFFECT OF INGESTION OF GLYCINE UPON URIC ACID OUTPUTS AND RETICULOCYTE COUNTS OF THE DALMATIAN COACH DOG

	RETICULOCYTES PER C MM. OF BLOOD	DAILY UPRINE VOLUME C C	DAILY URIC ACID OUTPUT IN MG	FED
Feb 14	35,700	200	320	
15	39,760	310	352	
16	27,150	450	328	
17	27,400	275	312	3 grams glycine
18	52,110	640	727	6 grams glycine
19	6,020	400	400	
20	11,640	280	320	
21	22,520	510	500	
22	10,400	270	432	

TABLE II
EFFECT OF INGESTION OF UREA UPON URIC ACID OUTPUTS AND RETICULOCYTE COUNTS OF THE DALMATIAN COACH DOG

	RETICULOCYTES PER C MM. OF BLOOD	DAILY UPRINE VOLUME IN C C	DAILY URIC ACID OUTPUTS IN MG	FED
Dec 31	33,920	810	405	
Jan 1	17,040	450	300	
2	42,280	380	432	75 grams urea
3	41,020	1315	1060	
4	34,710	350	400	
5	51,030	360	320	
6	34,980	350	400	

time when the base level was 377 mg. There was no subsequent reticulocytosis, and hence increased elimination of uric acid is again indicated (Table II).

Soda Bicarbonate—A dose of 20 gm. of sodium bicarbonate also produced a marked diuresis and with it flushed out a large quantity of uric acid. A volume of 2380 cc. carried with it 1260 mg. uric acid. This occurred on a low blood count of 3710,000 on an actively regenerating marrow, and hence the influence on reticulocyte count cannot be determined directly.

Ammonium Chloride—A series of consecutive doses of ammonium chloride showed somewhat similar results. Six grams given May 27 produced 830 cc. of

urine and 744 mg of uric acid, 16 gm the following day produced 2075 cc of urine and 1208 mg of uric acid, 8 gm on the next day produced 650 cc of urine and 750 mg of uric acid. This was in a period of active regeneration with a blood level of 3,520,000 on May 26 to 4,860,000 on May 30. During this period no marked rises in reticulocytes occurred which might be attributed to the action of the salt, the level being maintained at 14,000 to 41,000.

One dose of lithium citrate and one dose of uva ursi were also given but without any diuretic effect.

Caffeine—Eight doses of caffeine were administered under a series of conditions. The first dose of 450 mg produced a urine volume of 550 cc and 550 mg of uric acid. The second dose was evidently subminimal, since the volume dropped to 315 cc and 168 mg of uric acid. The third dose, given three days later in three portions of 300, 300 and 450 mg, resulted in a urine volume of 475 cc and 672 mg of uric acid. The fourth dose was 600 mg, given on a blood level of 4,000,000 and just after a spontaneous uric acid peak of 675 mg, due to

TABLE III
EFFECT OF INGESTION OF CAFFEINE ON URIC ACID OUTPUTS BY THE DALMATIAN COACH DOG

FED		DAILY URINE VOLUME IN CC	DAILY URIC ACID OUTPUT IN MG	R.B.C.
Jan	9	450 mg caffeine	460	368
	10	300 mg caffeine	550	550
	11		315	168
	12		470	248
	13	1050 mg caffeine	325	288
	14		475	632
	23		675	675
	24	600 mg caffeine	500	400
	25		300	344
	29		370	424
	30	1200 mg caffeine	600	650
	31		1470	1170
	Feb 1		440	584
	26		400	456
	27	1200 mg caffeine	560	448
	28		1750	439
Mar	7		600	480
	8	1200 mg caffeine	570	648
	9		1670	835
	10		290	376
23*			875	776
	24	1200 mg caffeine	915	808
	25		1460	1064

*Period of placental feeding hence the high uric acid base level

active erythrocyte regeneration. This may explain the drop to 344 mg of uric acid with a volume of 300 cc after the caffeine. The next four doses are of 1200 mg caffeine each, and marked diuresis was obtained in each case. The fifth dose, on a low blood count of 4,000,000 and an active marrow, brought out 1170 mg of uric acid in a urine volume of 1470 cc. The sixth dose, given on a blood count of 6,000,000 and after three high daily outputs due to the ingestion of nucleic acid, produced a marked diuresis of 1750 cc but with an extremely low

uric acid content of 439 mg. The seventh dose, on a blood level of 5,870,000 and a low reticulocyte count, resulted in a urine volume of 1670 cc and 835 mg of uric acid. The eighth dose was followed by 1460 cc of urine and 1064 mg of uric acid, but the last value is of no significance since it occurred in a period of active regeneration, low blood count, and placental feeding.

It is of interest to note that in the experiments reported by Myers and Wardell (1928) initial doses of caffeine always acted as diuretics and the largest outputs of uric acid came down with the largest volumes. They seem to favor the hypothesis that the caffeine is converted into uric acid, principally because of the fact that while theobromine is a good diuretic, it failed to increase the uric acid outputs in their four tests. An examination of their data shows that the dosage used failed to act as a diuretic. Their tests with euphryn show definitely that fluid intake and output were increased and that the uric acid increased proportionately. Our data seem to indicate that caffeine increases uric acid outputs by diuresis since the amounts are correlated with the condition of the marrow at the time.

The only outstanding fact against this explanation is the observation of Clark and Lorimer (1926) that the blood uric acid rises simultaneously with increased outputs under caffeine feeding.

The reticulocyte behavior under the conditions of caffeine administration throws further light on this explanation, and at the same time presents some evidence as to the mechanics of erythrocyte delivery.

Reticulocytosis Induced by Caffeine—In six of the eight tests of caffeine, a marked reticulocytosis was produced. The increases, however, occurred on the day subsequent to the administration of the drug, and in this way differed from the spontaneous rises observed after hemolysis and hemorrhage, which followed at intervals of from three to four days after uric acid peaks as reported in an earlier paper (Krafka, 1930).

The first dose of 450 mg caffeine, brought out 64,020 reticulocytes at a time when the prevailing level was 38,010. With a higher prevailing level, caffeine flushes out proportionately more reticulocytes. Thus the fifth dose of 1200 mg brought out 304,850 reticulocytes, when the count on the previous day was 149,720. The values for the other doses are given in Table IV. Proportional increases of 1.7 to 3.5 times were observed. The second and fourth doses present exceptions.

TABLE IV
EFFECT OF INGESTION OF CAFFEINE UPON THE RETICULOCYTE COUNTS IN THE DALMATIAN COACH DOG

CAFFEINE			PETICULOCYTE COUNTS			COEFFICIENT OF INCREASE
	ORAL DOSE IN MG	PREVAILING	AFTER CAFFEINE SUBSEQUENT			
Jan	9	450	38,010	64,020	39,500	1.7
	10	300	64,020	39,500	33,480	
	13	1050	28,900	75,140	34,500	
	24	600	100,500	104,000	98,560	2.5
	30	1200	149,720	304,850	55,480	
Feb	27	1200	31,400	77,550	12,240	2.0
Mar	8	1200	17,610	63,000	5,400	2.5
	24	1200	177,930	287,190	78,820	3.5
						1.8

It will also be noted from Table IV, that a plethora followed the increase. This is particularly evident in the massive 1200 mg doses. The fifth dose showed a drop to 56,480 from a preexperimental level of 149,720.

The data may be interpreted as due to the flushing action of the caffeine by increasing the vascular pressure. The entire reticulocyte crop is released into the general circulation instead of being retained for the maturing period of three to four days. A subsequent plethora follows.

These observations are of considerable interest in connection with the mechanics of erythrocyte delivery. Duncker, Duncker and Lund (1922) have shown that it is impossible to wash out nucleated red cells by increased mechanical pressure. In our eighth test with caffeine a check was made on this point, and it was found that there was no increase in the relative number of circulating normoblasts, although the reticulocyte count rose from 155,930 to 287,190. There is thus a marked observed difference in the mechanics of the delivery of these two types of cells.

This point is of such interest that the test was repeated on a series of ten rabbits. The data are given in Table V.

It is apparent from Table V that caffeine increases the reticulocyte counts, particularly after the regenerative process has begun after hemolysis. This point is well brought out in the test of June 11, 1931. Counts were made in the morning, caffeine administered at noon, and recounts were made in the afternoon, with increases consistent throughout.

The increases are not marked or regular on an inactive marrow, a fact also observed in the Dalmatian hound.

Atophan (Phenylcinchonic Acid)—Since this drug has been used rather extensively in gout, its relation to uric acid outputs has been widely studied. Our two tests failed to show anything of significance. An initial dose of 250 mg given at a time when the erythrocyte count was 6,100,000, reticulocytes at 0.3 per cent and uric acid at 560 mg, resulted in a subsequent R B C of 5,880,000, reticulocytes 0.2 per cent and uric acid 664 mg. A second dose of 750 mg failed to change the reticulocyte count or materially influence the uric acid output, the values being 584 mg before administration and 500 mg after.

Bilirubin—From time to time, the theory has been developed that the by-products of hemoglobin destruction may act in the capacity of erythropoietic agents, capable of maintaining the nice balance between destruction and production of erythrocytes. Veizal and Zih (1929) claim to have obtained marked increases in the red cell count by the oral administration of small doses of bilirubin and biliverdin. This observation was of such interest in an alternate hypothesis developed by the author, that a test of bilirubin was made, checking not only the red cell count but also the reticulocyte count and the uric acid values.

The initial dose of 3.5 mg of bilirubin was given when the erythrocyte count was 5,210,000, reticulocytes 0.4 per cent, uric 310 mg. The subsequent day, the red cell count was 4,870,000, reticulocytes 0.5 per cent and the uric acid output 288 mg. A second dose of 3.7 mg of bilirubin was given with the R B C at 4,700,000, reticulocytes at 0.1 per cent and uric acid at 424 mg. The result was

TABLE V
EFFECT OF CAFFEINE ON THE RHEUMATOID COUNT IN RABBITS

RABBIT NO	NO 1	NO 2	NO 3	NO 4	NO 5	NO 6	NO 7	NO 8	NO 9	NO 10
5/21	5,150,000 14	4,710,000 11	5,150,000 10	5,470,000 12	5,110,000 27	5,310,000 27	4,280,000 17	5,690,000 08	5,380,000 26	5,150,000 13
5/22	4,950,000 09	5,110,000 29	5,500,000 10	5,530,000 25	5,360,000 19	5,070,000 22	4,920,000 08	5,490,000 02	4,780,000 24	4,980,000 21
5/23	5,110,000 19	4,980,000 13	5,180,000 17	5,200,000 07	5,200,000 22	5,100,000 22	4,120,000 22	5,100,000 08	5,270,000 13	5,170,000 21
5/26	9 50 11 15 A M	50 mg caffeine citrate in water intravenous	Counts 11 20 1 12 1 M							
5/29	5,200,000 12	5,360,000 27	5,250,000 19	5,040,000 21	4,920,000 31	4,990,000 23	4,460,000 17	5,080,000 22	5,190,000 12	
5/28	9 00 10 00 A M	100 mg caffeine citrate by mouth	Counts 2 35 1 14 P M							
6/3	5,380,000 17	5,190,000 29	5,310,000 23	5,310,000 20	5,360,000 20	4,690,000 17	5,220,000 20	5,400,000 34	4,750,000 15	5,110,000 22
6/7	9 00 10 00 A M	100 mg caffeine (plain) by mouth	Counts 3 00 1 10 1 M							
6/7	5,650,000 24	5,380,000 19	5,560,000 11	5,710,000 35	5,710,000 17	5,080,000 19	5,710,000 34	5,950,000 18	4,970,000 15	5,290,000 25
6/7	9 30 10 00 A M	50 mg phenylhydrazine HCl by mouth								
6/8	4,780,000 17	4,660,000 28	4,560,000 24	4,360,000 26	4,600,000 12	4,660,000 27	4,450,000 29			
6/9	10 00 A M	50 mg caffeine orally	(Counts at 2 30 1 00 P M)							
6/9	4,150,000 90	4,700,000 86	4,540,000 66	4,000,000 173	3,380,000 97	3,870,000 78	3,200,000 82			
6/10	11 00 A M									
6/10	3,160,000 16	4,900,000 24	4,240,000 27	3,300,000 93	3,450,000 59	4,360,000 34	3,010,000 70			
6/11	10 00 A M									
6/11	3,780,000 18	3,660,000 56	4,510,000 18	3,540,000 103	3,410,000 12	4,020,000 57	3,310,000 67			
	(50 mg caffeine orally at 12 00 N	Counts 2 00 3 00 P M)								
	10 9	88	100	222	158	77	125			

insignificant since the erythrocytes were 5,100,000, reticulocytes 0.7 per cent and the uric acid level 320 mg. A third dose of 3.6 mg. gave similar results.

Verzár and Zih do not report reticulocyte counts in their work and until marked increases in this group of cells are demonstrated, bilirubin cannot be considered an active hematopoietic agent.

Xylose—As a corollary to the hematogenous theory of the origin of endogenous uric acid, a series of tests were carried out on the by-products of nuclear destruction in an attempt to find a factor which might control the production of erythrocytes by its varying concentrations. Uric acid was the first substance tried. A massive dose of 1500 mg. was given a patient with a severe secondary anemia without materially improving the red cell count or the reticulocyte count.

Larsell, Jones and Phillips (1927) have reported hematopoietic effects from the intravenous injection of nucleic acid and nuclear extractives in both rabbits and human beings. Dakin, Howe and West (1931) have identified the active substance in liver extract as a hydroxyproline. It seemed possible then, that somewhere in the splitting up of the nucleoprotein of the extruded normoblast nucleus, an active agent might be found. Of the three principal derivatives of nucleic acid, phosphoric acid seemed the least promising, since its concentration in the blood may be varied by so many independent processes. The pentoses, purines and pyrimidines remained, and two of these have been tested. On the basis of the report by Dakin, Howe and West that the precipitate containing both hypoxanthine and pentose from liver extract were inactive in the treatment of pernicious anemia, and that the active substance is free from pyrimidines, our own limited tests seem to indicate that nuclear derivatives are not concerned directly with the erythropoietic mechanism.

A test of pentose was carried out on the Dalmatian hound, by first depressing the erythrocyte count by hemolysis with phenylhydrazine and then feeding 5 gm. doses of xylose, observing the regenerative rate as shown by the erythrocyte and the reticulocyte counts. This was not in excess of regenerative rates spontaneously observed for the same animal in earlier tests. The data are given in Table VI.

The xylose was from cottonseed hulls purified by three recrystallizations by Dr. C. H. Maryott.

It is of interest to note that Madders and McCance (1930) report that the ingestion of 5 gm. doses of xylose or arabinose have no effect on the uric acid outputs in man.

Adenosine Compounds—Drury and Gyorgyi (1931) have extracted a substance from freshly minced bullock's heart by 5 per cent trichloroacetic acid which has a marked action on the heart of the dog when injected into the circulation. They have identified this substance as adenosine. Using the same method of extraction, we have checked the effect on reticulocyte counts in two rabbits, but without showing any activity on the marrow. We further checked the activity of the extract on a cat under urethane anesthesia and obtained a sharp fall in blood pressure upon the injection of 1 cc. The right femoral artery was ligated before the injection, the marrow of the right and left femora was fixed in formalin and sections prepared. No observable differences in the marrow of the two legs were found.

These observations are in keeping with those of the authors cited above that subcutaneous doses of 50 mg daily had no effect on the erythrocyte counts in guinea pigs

TABLE VI

EFFECT OF INGESTION OF XYLOSE UPON THE RETICULOCYTE COUNTS IN THE DALMATIAN COACH DOG

	ERYTHROCYTE COUNT PER CMM BLOOD	PER CENT RETICULOCYTES	FED
May 12	4,710,000	0.1	
13	4,670,000	0.1	
14	5,120,000	0.2	
15	5,260,000	1.3	50 mg phenylhydrazine HCl
16	4,560,000	0.5	
18	3,970,000	1.5	
19	3,900,000	1.7	5 gm xylose
20	3,910,000	1.0	5 gm xylose
21	3,900,000	1.9	5 gm xylose
22	4,150,000	2.1	10 gm xylose
23	4,420,000	2.2	
25	4,500,000	0.5	
26	5,030,000	3.2	

SUMMARY

1 Glycine, urea, sodium bicarbonate, and ammonium chloride increase the daily outputs of uric acid primarily by their diuretic action

2 Caffeine markedly increases the output of uric acid apparently through its diuretic action although the amounts vary with the regenerative states of the marrow

3 Caffeine produces a marked reticulocytosis the degree of which is dependent upon the marrow activity. It differs in time from the spontaneous reticulocytosis in that it follows the immediate administration of the drug

4 Caffeine does not increase the number of circulating normoblasts while the reticulocytosis is produced, hence a marked difference exists in the mechanics of delivery of these two types of cells

5 Minute doses of bilirubin failed to act as hematopoietic agents as checked by reticulocyte counts

6 Xylose failed as a hematopoietic agent as checked by reticulocyte counts

7 Adenosine also failed as a hematopoietic agent as checked by reticulocyte counts and by the histologic picture of the marrow

8 The data are considered in their relation to the theory that relates endogenous uric acid to the extruded nuclei of the normoblasts

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GINGIVITIS CHEMOTHERAPEUSIS AS AN AID IN THE DIAGNOSIS AND TREATMENT*

PRELIMINARY REPORT

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A FRESHLY isolated bacteria will, when subjected to the action of various antiseptics either be killed completely (bacteriolysis), be retarded (bacteriostasis), or grow as on ordinary nutrient agar, or other media. The antiseptic coefficient of standard drugs has no relation to the different organisms as different strains of the same species react to the same agent in a markedly different manner.

Based on the fact that members of the same group of bacteria and even different strains of the same organism have a diversity of reactions to a standard chemical it is seen that they exhibit degrees of resistance which are independent of the type of drug used but more dependent on the phase of their development. They do not actually build up a so called "immunity" to the drug but they manifest a varying degree of penetrability to the reagents and develop a tolerance to it. This degree of tolerance is definitely associated with morphologic and attenuation changes. Consequently, in freshly isolated rapidly developing and dividing strains this tolerance is less than in those strains which have been or are exhibiting advanced phases of microbial dissociation. Just as bacteria develop a resistant or "R" form to the continual lysis of the bacteriophage, so does a strain of bacteria develop a similar type of resistance to the continued use of an antiseptic.

It is this fact which explains a condition very frequently found in the treatment of gingivitis. A definite point or plateau is reached in treatment and from which no amount of medication with previously successful antiseptics has any effect. The lesion settles into one of chronicity with possibly periodic exacerbations of an acute nature. Or as in the case of Vincent's infection there is frequent recurrence.

Chemotherapeusis is in reality the adaption of suitable chemicals or drugs to the treatment for elimination of diseases and disease condition. These drugs and antiseptics are the most standard preparations of various drug houses. They are sold and standardized in relation to their (1) antiseptic powers (2) penetrative ability, (3) safety and convenience in usage.

The preparations most frequently in use in oral hygiene and for the treatment of gingival infections are divided into three main groups (a) mercurials, (b) salts of other metals and (c) dyes. With these, a chemotherapeutic standard is used of two strong antiseptics whose coefficient of antiseptics has been accepted, one is phenol itself (in a final dilution of 1-1,000) the other is tricresol (final dilution of 1-2,000). These groups of germicides give us a range of the common

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antiseptics covering most of the solutions used in ordinary dental or medical practice for the treatment of gingivitis

The technic followed is the same one used and described by Keilty,¹ the only variation is in the concentration of the work done on the streptococci isolated from gingival cultures by anaerobic as well as aerobic methods. Keilty's results have been so uniformly good that any variation would only slow up the treatment. The entire work depends on a cooperation between the laboratory and the dentist to give a maximum of efficient diagnosis and treatment.

Technic —

A Two direct smears are made for staining

- 1 For the usual stain by Gram's method,
- 2 For differential stain if wanted

B A suspension of the material, fresh, in salt solution for a dark field examination or with simple oil immersion lens, to note motility, character of the spirochete, protozoa, type and number of organisms

C A suspension in Schaudinn's fluid for ameba present

D A blood agar plate is inoculated for media control and initial culture from which subcultures are made for further bacterial identification

E Tubes of the special antiseptic agar are inoculated by stroke and stab to determine the degree of growth allowed by the different antiseptics

The antiseptic tubes are all made up as follows. Nutrient agar is melted and flaked in 50 c c lots, to this is added 2 per cent blood collected aseptically and 0.5 c c of a 1-100 dilution of the antiseptic to be tested. This gives in the final dilution an antiseptic agar of 1-10,000, in all flasks. This is then tubed in small tubes and used directly after slanting. The standards (phenol and tricresol) are made up in corresponding proportions to give the final dilutions as desired.

The following preparations are the ones made up as being the ones most commonly used by contributing dentists. We have discarded some and are adding others. For example, antiseptics which persist in showing growth in a dilution of the antiseptic of 1-10,000, and in dilutions of greater concentrations, which generally show growth are not suitable clinically where the dilution in the mouth is greater in a few moments than that which we are using in each test tube. Among those which gave consistently poor results are S T 37, and mercuriochrome. These have been discarded from the set up and we are confining the test to eleven antiseptics. They are

Mercurials	{ Metaphen (Abbott Laboratories) Merthiolate (Eli Lilly) Mercuriochrome (discarded)
Salts of metals Oxidizing agents	{ Copper sulphate Chromic acid Argyrol Iodine, acetone, glycerine (Prinz Formula)
Aniline dyes	{ Acridiolet Gentian violet
Standards	{ Tricresol Phenol

TABLE I
22 STRAINS OF ORGANISMS INHIBITED BY ANTISEPTICS

ORGANISMS		MERCURIALS		DYES		APGY POL
		MFTA PHEN	MERTHI OLATE	GENTIAN VIOLET	ACPI VIOLET	
Gram negative rods	<i>B coli communis</i>	G	NG	G	NG	G
	<i>B coli communior</i>	G	NG	G	G	G
	<i>B lactis aërogenes</i>	G	NG	G	G	G
	<i>M catarrhalis</i> , 3 strains	3 NG	2 NG	G	1 NG	G
Gram negative cocci	<i>B mucosus capsulatus</i>	NG	NG	G	G	G
	<i>S salivarius</i> (2 strains)	2 NG	2 G	2 G	2 NG	G
	<i>S progenes</i>	2 NG	3 G	3 G	3 NG	1 G
	<i>Strept subacidis</i>	G	NG	G	G	G
Streptococci	<i>S Hemolyticus</i> I	G	G	G	NG	G
	<i>S fecalis</i>	G	G	G	NG	G
	<i>S anginosus</i>	G	G	G	NG	NG
	<i>S mitior</i>	G	G	G	NG	NG
	<i>S equinus</i>	G	G	G	G	G
	<i>S aureus hemolyticus</i> (3 strains)	G	3 NG	1 NG	2 NG	G
Other gram positive cocci	<i>M tetragenus</i>	G	G	G	NG	G
	Pneumococci	G	NG	G	NG	G
	Diphtheroids	G	NG	G	NG	G

There is a definite relationship between the degree of infection and the type of organism present. In the most severe infections, there is a predominance of streptococci, and these respond less to treatment than do the other types of infection. Gram negative organisms are less difficult to clear up than the gram-positive ones. The opinion has been prevalent that the gram-positive organisms respond better to drugs which have as a basis one of the coal tar products, i.e., the dyes, and that the gram-negative organisms respond best to mercurials. This is not wholly in accord with our findings. The streptococci respond equally well to the mercurials as to the dyes, but there is a slightly greater affinity for the gram-negative rods and the mercurials. A mixed flora, however, might react differently to two of the individual strains and require still a third antiseptic to act on the combination of the strains.

Table I shows the results of the isolation of single strains of bacteria by culture. When isolated pure they were then put through all of the chemotherapeutic media. The streptococci are classified according to Holman,² based on their fermentation reactions. All the organisms were isolated from gingival sulci except the strains of the colon group which were obtained from cases of mucous

colitis The antiseptics which gave retarded growth are not included in the table, growth however feeble was recorded as being equal to no inhibition

In inoculating slants, two main methods were tried in order to get a method for comparative results The first was to inoculate a tube of Rosenow's deep brain broth from a pus pocket After twenty-four hours' incubation a loopful of the broth culture was transferred to the antiseptic tubes and incubated over night This gave varying results but there was doubt as to all of the organisms present in the pus pocket surviving, as it is well known that there is inhibition of one type of organism by another Therefore slants were inoculated directly from the gingival sulcus or pocket In badly infected cases this generally gave the same amount of material transferred to each tube, generally a loopful Following is the result of fifty cases so inoculated

TABLE II
FIFTY CASES OF GINGIVITIS, CHEMOTHERAPEUTIC RESULTS

ANTISEPTIC	RETARDED	PER CENT	NO GROWTH	PER CENT
Chromic acid	4	8	0	0
Copper sulphate	2	4	0	0
Merthiolate	6	12	17	34
Mercurochrome	0	0	0	0
Metaphen	12	24	10	20
Argyrol	0	0	2	4
Gentian violet	4	8	0	0
Acriviolet	4	8	19	38
Phenol	14	28	10	20
Tricresol	14	28	6	12
I A G	3	6	4	8

When growth occurred the antiseptic was considered as being a failure, even though the growth was markedly restricted In Table II a report is made of this retardation but is merely placed there as a matter of record for comparative purposes

The percentage of nongrowth produced by the different cultures of the gingiva should determine the percentage of its efficiency Table III shows the percentage of each of the antiseptics used in relation to their bacteriolytic efficiency

TABLE III
PERCENTAGE CHART OF ANTISEPTICS USED

SOLUTION	PER CENT OF EFFICIENCY
Acriviolet	38
Merthiolate	34
Metaphen	20
Phenol	20
Tricresol	12
Iodine, Acetone, Glycerine	8
Argyrol	4
Chromic acid	0
Copper sulphate	0
Gentian violet	0
Mercurochrome	0

Gingival cultures show a diversified bacterial flora. In Table IV there is a high percentage of streptococci and *M. catarrhalis*. The unexpectedly large percentage of moulds found is noteworthy.

TABLE IV
BACTERIAL INCIDENCE IN 100 CASES OF GINGIVITIS

ORGANISMS	PER CENT OF CASES	ORGANISMS	PER CENT OF CASES
<i>M. tetrigenus</i>	24	<i>S. aureus</i>	44
<i>B. mucosus capsulatus</i>	19	<i>S. albus</i>	6
Diphtheroids	29	<i>S. citreus</i>	1
Pneumococci	76	<i>S. hemolyticus</i>	17
<i>B. influenzae</i>	9	<i>S. nonhemolyticus</i>	15
<i>M. buccalis</i>	2	<i>S. infrequens</i>	12
Streptothrix	48	<i>S. salivarius</i>	33
Leptothrix	81	<i>S. pyogenes</i>	12
<i>M. catarrhalis</i>	73	Protozoa*	75

**Trichomonas buccalis* 36 per cent.
Endameba buccalis 39 per cent.

The application of the germicide is an important factor. It is immaterial how it is applied if one is certain that the bacteria and the germicide are in contact. For this purpose a flexible rubber vacuum cup has been recommended. This holds a sufficient quantity of solution to be time-saving in application. There is no doubt also, that there are benefits derived from the vigorous massage given by the use of this cup. Pus pockets of depth are reached by the use of a bent, blunt platinum needle with a syringe. The method of application is secondary if the fact be borne in mind that any germicide must be in contact with the organisms to be effective.

In treatment the alternate use of the suggested drug has been combined with arsphenamine in some form or other. This is used to clear up the spirochetes which we have found to be present in 86 per cent of all normal mouths, and in 98 per cent of all those infected with one or more of the streptococcus group. It is doubtful whether the type of arsenical can be used indiscriminately, but the results with neoarsphenamine and sulpharsphenamine in glycerine, applied topically, have been remarkably good when used with the various antiseptics. These arsenic preparations are definite spirocheticides and clear up the spirilla present.

SUMMARY

Chemotherapy is definitely indicated in gingival infections. The cooperation of the laboratory and dentist is necessary in suggesting the proper antiseptic to use in treatment of gingival infections such as streptococcal gingivitis, ulceromembranous gingivitis, pyorrhea, etc.

Gingival infections due to gram-negative organisms respond to treatment better and more rapidly than do those due to gram-positive organisms. There seems to be no basis for the idea prevalent in medical minds that the gram-positive organisms respond more successfully to the dyes than to the mercurials.

Gingival cultures show a diversified bacterial flora with a large percentage of moulds and 89 per cent of streptococci present in lesions. For chemotherapeutic diagnosis inoculation by stroke and stab is necessary and direct inoculation gives the most accurate results. Methylolite, metaphen, acriviolet, and phenol were found to be the four antiseptics of highest bacteriolysis of eleven used in a series of fifty cases.

In treatment the use of two or more of the bacteriolytic antiseptics used alternately and in combination with asphenamine has been found to give the most satisfactory results and with fewer recurrences.

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MERTHIOLATE AS A SKIN DISINFECTING AGENT*

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INTRODUCTION

MANY studies have been published upon the use of various germicides as human skin sterilizing agents^{1, 2, 3, 4, 5, 6, 7, 8}. In some instances in the literature on this subject, artificial conditions have been introduced, such as animal skin disinfection tests, the use of laboratory cultures as well as certain contaminated materials artificially applied to the skin, and experiments dealing only with the very superficial bacteria associated with the skin. Such tests do not necessarily indicate the true effectiveness of a germicidal agent against the natural skin flora of the human. In other instances the technic employed has been more or less tedious and complicated, seemingly practical only in certain conditions where the time element has not been an important factor.

The ideal germicide for skin disinfection should be effective in causing practically complete destruction of not only surface skin bacteria but also those situated in the deeper layers. Antiseptic action should be maintained for several hours after application. In addition, the ideal germicide should not injure the most sensitive skin, and may best fulfill its function if it directly stimulates tissue cell growth and healing.

Merthiolate (sodium ethyl mercuri thioarsalicylate), an organic mercurial compound, seems to fulfill the requirements of a satisfactory disinfectant for the skin. This compound has been shown to possess active germicidal properties maintaining its effectiveness in the presence of media most nearly resembling the tissues, such as serum agar and white clot or fibrin agar⁹. It is readily soluble, possesses definite penetration properties, and does not precipitate serum proteins. Merthiolate has a low degree of toxicity for animals and human beings, does not hemolyze red blood cells, and does not injure sensitive bacterial antigens and antibodies^{9, 10, 11}. It has been found to stimulate tissue cell growth and healing¹². Merthiolate has also been reported to approach the ideal germicide very closely in tests utilizing bacteria and living tissue growing *in vitro*¹³. In these new tests, which give most important information as to the real value of a germicide combining as they do readings on the germicidal value and effect on living tissue, merthiolate has a rating of 0.9, and phenol a rating of 0.2, as against a minimum rating of 1.0 for a theoretically ideal germicide. The close approach of merthiolate to the ideal in these tests indicates that a high degree of efficacy should be realized in tissue antiseptics.

Reimann¹⁴ has reported that some individuals display a sensitiveness to thio compounds which is characterized by reddening of the treated area and the ap-

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pearance of small papules and vesicles. Since merthiolate is a derivative of a thio compound, the occasional erythema noted in a few highly sensitive persons following the use of unstabilized aqueous merthiolate solution may in part be attributable to this factor. Studies on procedures which bring about stability of solutions and overcome this possible tendency as well as the possibility of infrequent mercurial dermatitis in particularly sensitive persons will be published separately. These studies, which are to be reported soon, show that by rather simple stabilization methods applied to the solvent, merthiolate solution can be so prepared that it may be applied in 1:1000 dilution as wet dressings for twenty-four hours or longer without causing irritation in highly sensitive individuals who previously reacted after one minute exposure to the unmodified solution. Such stabilized merthiolate solutions¹² retain their healing properties. The merthiolate solutions used in the work reported in this paper included stabilized solutions which cause no irritation in either ordinary or hypersensitive subjects.

The work reported here consists of experiments indicating the comparative potency of merthiolate in skin disinfection procedures under conditions approaching as nearly as possible those encountered in ordinary clinical practice. A simple, rapid technique was employed. The organisms dealt with consisted in all cases of those normally resident upon or within the human skin. These included gram-positive cocci and various mixtures and proportions of gram-positive and gram-negative bacilli. Tests were carried out in such a way as to determine the value of merthiolate not only in the preoperative preparation of the human skin, but also in first aid antisepsis. Tincture of iodine U.S.P. was used as a control germicide so that results would be comparative. The merthiolate preparations used included both colored and uncolored 1:1000 aqueous solutions, and colored and uncolored 1:1000 alcohol acetone aqueous solutions.

EXPERIMENTAL

General—The technique used in this work unless otherwise stated is as follows. Human skin areas of about four inches in diameter were treated with soap and water and then shaved. The skin was next thoroughly scrubbed with tincture of green soap and water by means of a gauze sponge. The area was then rinsed with sterile water and dried with a sterile sponge. At this point a culture was taken from the center of the treated area by rubbing the skin vigorously with a sterile swab wet with broth. This swab was rubbed up in a tube of 10 c.c. of fresh veal infusion broth. Following this a gauze sponge wet with the germicide was rubbed evenly over the area for ten to fifteen seconds. After a one minute exposure the excess of germicide was rinsed off with sterile water and the area quickly dried with a fresh sterile gauze sponge. Bilaterally symmetrical skin areas were used, one germicide being placed on one side and the other on the opposite side. Immediately after a one minute exposure a surface culture was taken by vigorously rubbing a portion of the treated area with a sterile swab wet with broth. This swab was rubbed up in a tube of 10 c.c. of fresh veal infusion broth. Deep skin cultures taken at the same time as the surface skin cultures were made as follows. An area of skin about one-eighth inch by one-half inch was scraped with a sterile scalpel until capillary bleeding occurred.

These scrapings were planted to broth tubes. In addition, immediate subcultures to blood agar plates were made from both the surface and the deep skin cultures, using 1 c c amounts. After twenty-four hours' incubation at 37° C both the surface and deep skin cultures were subcultured in 1 c c amounts to fresh 10 c c broth tubes. All cultures were read after a further incubation of forty-eight hours, i e, three days after the tests, and may be listed for convenience as follows:

1 Control culture made after cleansing the skin but before application of the germicide

2 Surface skin broth culture made one minute after application of the germicide

3 Subcultures to blood agar plates made from No 2 within one-half hour

4 Subcultures to broth tubes made from No 2 after twenty-four hours' incubation

5 Deep skin broth cultures made one minute after application of the germicide

6 Subcultures to blood agar plates made from No 5 within one-half hour

7 Subcultures to broth tubes made from No 5 after twenty-four hours' incubation

Subcultures were made as indicated above to rule out the possibility of inhibition from an excess of germicide being carried over into the cultures and interfering with the determination of the actual killing of the bacteria. This possibility had been considerably minimized by the rinsing of germicide treated skin areas previously described. The regions selected were distributed quite evenly over the body and included those areas frequently subjected to surgery. Twelve white and three colored individuals offered themselves as subjects for these studies. The tests were carried out during the months of May, June, and July, and various degrees of skin hygiene were encountered.

TABLE I

SUMMARY OF COMPARATIVE SURGICAL SKIN DISINFECTION TESTS WITH MERTHIOLATE AQUEOUS 1:1000

GERMICIDE	NO OF SKIN TREATMENTS AND CULTURES	SURFACE SKIN CULTURES			DEEP SKIN CULTURES		
		STERILE	CONTAMINATED	PER CENT STERILE	STERILE	CONTAMINATED	PER CENT STERILE
Merthiolate, aqueous 1:1000	47	47	0	100	43	4	91.4
Control cultures before application of Merthiolate	47	0	47	0			
Tincture of iodine, U S P	40	34	6	85	33	7	82.5
Control cultures before application of tincture of iodine	40	0	40	0			
Distilled Water Control	10	0	10	0	0	10	0

Presurgical Skin Disinfection Tests with Merthiolate, Aqueous 1 1000—Table I gives a summary of the skin disinfection results obtained with merthiolate, aqueous solutions 1 1000. Because of preliminary washing with soap and water, the aqueous solutions wet such areas adequately. Clear aqueous solutions of merthiolate 1 1000 as well as aqueous solutions colored with various dyes were equally effective, and this agrees with our test tube experiments not reported here. Of 47 merthiolate applications, the surface skin was sterilized in all cases (100 per cent), and the deep skin was sterilized in 43 tests (91.4 per cent). Of 40 tincture of iodine applications under exactly comparable conditions the surface skin was sterilized in 34 cases (85 per cent), and the deep skin was sterilized in 33 tests (82.5 per cent). In 7 of these tests, control tincture of iodine applications were not made, due to the delicate nature of the regions selected. Control tests consisting of washing of similarly prepared areas with sterile water for one minute instead of germicide, resulted in entire failure to sterilize the skin. In all instances the control cultures taken after scrubbing with soap and prior to application of the germicidal agents were positive, showing that the scrubbing was not effective as regards skin disinfection.

TABLE II
SUMMARY OF COMPARATIVE SURGICAL SKIN DISINFECTION TESTS WITH MERTHIOLATE,
ALCOHOL ACETONE AQUEOUS 1 1000

GERMICIDE	NO OF SKIN TREATMENTS AND CULTURES	SURFACE SKIN CULTURES			DEEP SKIN CULTURES		
		STERILE	CONTAMINATED	PER CENT STERILE	STERILE	CONTAMINATED	PER CENT STERILE
Merthiolate, alcohol acetone aqueous 1 1000	57	55	2	96.4	52	5	91.2
Control culture before application of Merthiolate	57	0	57	0			
Merthiolate, alcohol acetone aqueous diluent control	43	19	24	44.1	8	35	18.6
Tincture iodine, U S P	43	43	0	100.0	36	8	83.7
Control culture before application of tincture of iodine	43	0	43	0			

Presurgical Skin Disinfection Tests with Merthiolate, Alcohol Acetone Aqueous 1 1000—Table II gives a summary of the skin disinfection results obtained with merthiolate, alcohol acetone aqueous 1 1000. In addition to the tincture of iodine control there was also included an alcohol acetone aqueous control, using the same formula for the mixture as was used in the merthiolate, alcohol acetone aqueous solutions. In order to delineate clearly the areas treated, the merthiolate solutions were colored with various dye substances which previous test tube experiments had shown did not modify the germicidal value of merthiolate. One of these solutions, colored with eosin and sodium fluorescein, proved very satisfactory of application, changing in color from yellow to pink upon dry-

ing Of 57 merthiolate alcohol acetone aqueous applications the surface skin was sterilized in 55 cases (96.4 per cent) and the deep skin was sterilized in 52 tests (91.2 per cent) Of 43 tincture of iodine applications the surface skin was sterilized in all cases (100 per cent), and the deep skin was sterilized in 36 tests (83.7 per cent) Of 43 control applications using the alcohol acetone aqueous diluent of merthiolate the surface skin was sterilized in 19 tests (44.1 per cent), and the deep skin was sterilized in 8 tests (16.6 per cent) In all cases the control cultures taken after scrubbing with soap and prior to application of the germicides were positive

Maintenance of Skin Antisepsis with Merthiolate—In the tests reported above, and in fact in nearly all work heretofore reported the immediate condition of the skin i. e. whether sterile or contaminated, has been the only question involved It seemed desirable to inquire into the duration of antisepsis inasmuch as a long period of antibacterial action is often desirable especially in cases involving trauma with devitalization of the tissues and in certain operative procedures

In order to determine the value of merthiolate in this connection two bilaterally symmetrical abdominal skin areas were prepared, as described previously, in each of ten adult subjects One area was treated with merthiolate,

TABLE III
COMPARATIVE MAINTENANCE OF SKIN ANTISEPSIS FOLLOWING USE OF MERTHIOLATE

GERMICIDE	NO OF TEST SUBJECTS	SKIN TESTS MADE AFTER	NO STERILE	NO CONTAM INATED	COMPUTED NUMBER SKIN BAC TERIA PLANTED IN TUBES PROV ING SKIN CONTAMINATED
Merthiolate, alcohol acetone aqueous 1:1000	10	15 min	9	1	40
	10	1 hour	8	2	20,250
	10	2 hours	8	2	10,200
	10	3 hours	8	2	10,80
	10	5 hours	9	1	150
Tincture iodine, U. S. P.	10	15 min	9	1	500
	10	1 hour	10	0	0
	10	2 hours	9	1	200
	10	3 hours	9	1	20
	10	5 hours	8	2	40 30

alcohol acetone aqueous 1:1000 and the corresponding area with tincture of iodine Surface skin cultures and deep skin cultures were taken at the end of five minutes and appropriate subcultures were made A sterile gauze dressing was then applied to each area and the subjects went about their usual work Culturing of each treated area was repeated after one two three and five hours During the time of the tests the temperature was above 90° F and some of the subjects perspired freely The results of these tests are summarized in Table III They indicate that both merthiolate and tincture of iodine maintain relatively good antiseptic action under rather severe conditions Those cultures taken after application of the germicide which proved positive originated from relatively few bacteria as compared to the enormous numbers of bacteria recovered from untreated skin Two of the ten subjects used in this experiment

showed toward the close of the five hour test period quite intense erythema in the areas treated with tincture of iodine. The merthiolate, alcohol acetone aqueous solution did not provoke any skin reaction. It was regularly noted that the abrasions made in merthiolate (aqueous or alcohol acetone aqueous) treated areas for deep skin cultures healed more rapidly than similar abrasions in the iodine treated areas. This phase of the work is the subject of a separate report.¹

Merthiolate in First Aid Skin Disinfection Procedures—Merthiolate, alcohol acetone aqueous 1:1000 and tincture of iodine, U.S.P. have been used in a number of tests upon the untreated skin of individuals, utilizing areas such as the dorsum of the hand and foot, shin, forearm, and upper arm. In these subjects previous washing with soap and water was omitted, and the tests did not involve trauma. It has been regularly found that the skin treated with either merthiolate or tincture of iodine is sterilized in a high proportion of cases. In those where contamination remained, the number of surviving organisms was infinitely small compared to those present in the normal skin. These results are in accord with those listed in detail above, showing the efficiency of merthiolate as a skin disinfecting agent.

SUMMARY

- 1 The effectiveness of merthiolate (aqueous and alcohol acetone aqueous) in preoperative and first aid skin disinfection is reported.
- 2 Artificial test conditions were avoided by
 - a The use of the natural flora of the skin as test organisms
 - b The employment of a technique as nearly similar as possible to that used in regular clinical practice
- 3 The tests indicate that merthiolate approaches the ideal germicide for skin disinfection procedures because of the following properties demonstrated under conditions comparable to actual clinical use
 - a High germicidal activity against surface skin organisms
 - b High germicidal activity against deep skin organisms
 - c Rapidity of skin disinfection
 - d Maintenance of condition of antisepsis over a considerable period of time
 - e Nonirritating to the most sensitive skin
 - f Freedom from vapors irritating to the eyes of the operators and attendants
 - g Promotion of healing of abrasions by actively stimulating tissue cell repair

We are indebted to Dr. G. H. A. Clowes, Director of Research, Eli Lilly and Company, for counsel and suggestions during the course of this study.

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TREATMENT OF RHEUMATIC FEVER WITH A MAGNESIUM CINCHOPHEN, MAGNESIUM OXIDE (MAGNEPHEN) PREPARATION*

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THE medicinal treatment of rheumatic fever has been limited chiefly to salicylates and combinations of the phenyleinchoninic acid group. These drugs in no way alter the course or duration of an attack, they do, however permit a more rapid convalescence by alleviating the joint pains, lowering the temperature, and also possibly by eliminating the intoxication.¹ It is now well established that even though the temperature approaches the normal level and there are no joint pains, activity of the rheumatic virus may be present under most adequate medication and evidences of such activity are leucocytosis, increased sedimentation time of erythrocytes and even the development of cardiac lesions.^{1 2} While most of the time salicylates and the various phenyleinchoninic preparations make the patient comfortable by relief of pain, the intoxication caused by these drugs offsets at times the advantages obtained from their use. The buzzing in the ears, headache, nausea, vomiting, and sometimes the diarrhea are most annoying and such symptoms may be produced even by small doses, depending on the sensitivity of the individual to the drug used.

To combat this disadvantage Lees³ suggested the administration of sodium bicarbonate with salicylates. He believed that their toxicity was reduced by the combination. This assertion, however, has not been substantiated by others. It has been the usual practice to use substitutes when evidences of salicylate intoxication were apparent. Unfortunately such substitutes were also without therapeutic value unless given in large doses which, too, were toxic. Hanzlik⁴ believes that the search for substitutes or derivatives of these drugs is futile. He is of the opinion that a better tasting and perhaps less soluble and less irritating combination may be evolved, but its effect may not be so marked as is noted by the use of the salicylates or cinchophen preparations. "Full therapeutic efficiency and toxicity go hand in hand" and—"absence of toxicity means absence of therapeutic efficiency," states Hanzlik.

While searching for a combination which would be less toxic and still have its full therapeutic effect, Barbour and Winter⁵ have found that magnesium salts potentiate the activity of salicylates, and members of the phenyleinchoninic acid group. These investigators found evidence that the magnesium salt of phenyl cinchoninic acid is less toxic for mice than the sodium salt. They also observed that the magnesium compound was a more efficient antipyretic in fevered rabbits, and furthermore the addition of magnesium chloride to the magnesium com-

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These tablets are known commercially as magnephen and were supplied to us through the courtesy of the Calco Chemical Company.

pound of phenyleinchoninic acid produced more marked onset of antipyresis and they expressed the view that it pointed to a temporary synergism of the compound

With such advantages claimed for a preparation, we were anxious to use it in the treatment of cases of rheumatic fever which were under our care. The magnesium compound of the magnesium salt of phenyleinchoninic acid was used. This was made up in tablets of $7\frac{1}{2}$ grains (0.5 gm.) each thus making its administration simple and enabling us to keep accurate records of the amount used. Each tablet contained 4 grains (27 gm.) of magnesium cinchophen and 3 grains (20 gm.) grains of magnesium oxide. One hundred grains (6.6 gm.) of the new preparation are actually equivalent to 45 grains (3.0 gm.) of phenyleinchoninic acid. Our series of cases is not very large. The observations, however, have been careful, the data complete and the results so promising and uniform that we felt justified in presenting these cases so that others may be encouraged to use these and other similar preparations.

There has been a tendency recently to avoid the use of cinchophen and its various combinations, because of its believed toxic action on the liver. Numerous publications both in this country and abroad have shown that a hepatitis and occasionally acute yellow atrophy of the liver result from the use of this drug. Barron⁶ has shown that even 15 grains (1.0 gm.) can bring on an attack of shock within two hours. His patient recovered. Evans⁷ reported three cases of hepatitis following the use of such preparations. Among the other numerous reports in the more recent ones of Sutton⁸ and Rabinowitz,⁹ Parsons and Harding,¹⁰ and Sherwood and Sherwood,¹¹ more evidence is adduced both from personal observations as well as complete reviews of the literature, as to the toxicity of these preparations and particularly their predilection for liver damage, often with fatal results. Churchill and Van Wagoner¹² produced cinchophen poisoning in dogs by using 27 times the therapeutic dose. These large doses produced acute gastric ulcers, and some liver damage, as was shown by the diminished liver function. Six of their animals died. When, however, the therapeutic dose of cinchophen was fed, even though the kidneys were artificially damaged by clamping the renal artery, no death resulted, but only slight transient manifestations of liver damage. Fishberg,¹³ fed therapeutic and even toxic doses of the drug to rabbits and was unable to find signs of liver damage either grossly or microscopically. He also administered a soluble cinchophen preparation to rabbits by vein and could not demonstrate pathologic changes in the liver. With reports that are not quite unanimous and knowing that the phenyleinchoninic group preparations have been and are still used extensively, we felt that their damaging effect is far from constant. It may be that certain individuals are sensitive to them, and it is in such cases that the hepatitis results. In other words it is not the drug but the patient who is at fault. The knowledge, however, that untoward results may be brought about by the use of the cinchophen group warrants most careful vigilance, and the earliest evidences of toxicity such as anorexia, vomiting, urticaria or jaundice dictate their immediate discontinuance. We have used a cinchophen compound in the treatment of our cases fully cognizant of the possible hazards and consequently most careful in our

observations and care. We insisted that our patients have ample carbohydrate in their diet. This we felt afforded some protection to the liver.

Even though we have treated seven cases of rheumatic fever, we are only reporting four in detail. The other three were treated when we were not very familiar with the drug and we felt unjustified in subjecting acutely ill patients to experiment when an established remedy was at hand. With the earlier cases, therefore, we were cautious in the administration of the new preparation as we knew nothing of the dosage or its by-effects on human beings. We administered large doses of salicylates to the patients when they were acutely ill and after their symptoms were considerably relieved we substituted the magnesium phenylmethoninic acid preparation. At first we used $7\frac{1}{2}$ grains (0.5 gm.) every four hours. Since no toxic effects were noted, we gradually increased the dosage to as high as 30 grains (2.0 gm.) every four hours. In our limited experience 75 grains (5.0 gm.) to 90 grains (6.0 gm.) per day were ample to produce therapeutic effects without concomitant toxicity. As we learned more about the preparation, we used it as soon as the cases were admitted, and the four cases we are presenting are the results of such observations. The cases here reported were in the acute phase of the disease. On admission each patient received the routine care and after a physical examination, blood examination for its count, sedimentation time and the retic index of the serum, the medication was started. The total dosage was distributed throughout the day at four-hour intervals. Our patients were kept on medication and in bed until all evidences of activity were gone. The temperature and pain were the first to respond. The patients then felt well and asked for certain liberties, stating that they were well enough to be up. Their requests were rejected as long as there was a leucocytosis and an increased sedimentation time. When, however, the temperature dropped, the pain vanished, and the latter two criteria approached normal limits, the medication was discontinued, the patient was allowed out of bed by degrees, and after a week or ten days of further observation without any medication, discharged.

CASE 1—(No. 1563) A male Chinese laundryman, aged thirty three, was admitted March 3, 1931. He complained of pains in the left and right hips, knees, ankles, shoulders, and wrists for four days previous to admission, the pains were accompanied by dyspnea and cough, and a purpuric rash appeared on both legs. No history of previous attacks was obtained and there were no contributory facts, either in his past or family history. Temperature 102° , respiration 22, pulse 136. He appeared acutely ill, was moderately dyspneic and slightly cyanosed. The teeth and gums were in poor condition, the throat injected, and the tonsils inflamed. The heart showed a diffuse heaving impulse and appeared enlarged to percussion. The sounds were forceful, totally irregular and rapid. In the pulmonic area a rough systolic murmur was heard. Blood pressure 110/66. The liver edge was felt below the costal arch, the spleen was not felt. Over both legs there were numerous ecchymotic areas, varying in size from that of a pinhead to that of a large pea. There was tenderness in the joints but no swelling or redness. The blood Wassermann and blood cultures were negative. Coagulation time seven minutes, bleeding time two minutes, clot retraction normal. RBC 4,660,000, HbO 70 per cent, WBC 6,800, with a practically normal differential count. Sedimentation time was 30 per cent. The electrocardiogram showed auricular fibrillation, poor electromotive force and small upright T waves. The patient was immediately placed on "magnephen," 75 grains (5.0 gm.) daily. The pain and tenderness disappeared within three days, and incidentally, the purpuric rash began to fade. The temperature dropped to normal on the fourth

day and the heart rate was 90. With the slower heart rate, evidences of mitral stenosis were made out. For seven days he was comfortable. He then developed a red throat and an abscess of the gum above the upper left second bicuspid. This was incised, following which the temperature dropped to normal, remaining at this level until discharged. March 28, twenty five days after admission, medication was discontinued as the temperature had been normal for ten days and the leucocyte count was 7,000 with the sedimentation time of 5 per cent, and on discharge, April 9, it was again 5 per cent. This patient had ingested 1,875 grams (1250 gm) of the preparation and at no time were there symptoms of toxicity and the icteric index on discharge was 5.

CASE 2—(No 15611) An Italian boy thirteen years old was admitted on February 27, 1931, complaining of pains in the knees, ankles, wrists, and small joints of the hands for ten days. The present attack was initiated by a sore throat and cold in the head. There was a history of a previous attack of rheumatic fever three years before and the patient was under observation in the out patient department. Other than a systolic murmur on his former admission, no abnormalities of the heart were recorded. He had had measles in early childhood and a tonsillectomy when three years old. The physical examination revealed a well nourished and well developed pale boy. The previously observed systolic blow at the cardiac apex was noted, the liver was just palpable and there were swelling and tenderness in both ankles and the right third metacarpophalangeal joint. On admission white count was 12,500 with 72 per cent polys. The Wassermann was negative and the urinalysis revealed a trace of albumin. He was treated with sodium salicylate, 90 grams daily, as his temperature was 101° and he was obviously in the acute phase of the disease. We preferred salicylates at first here, since we were eager to bring about symptomatic relief rapidly and did not feel justified in experimenting with a drug about which we knew little. On the third day he was practically symptom free, on the fifth day the temperature dropped to normal and on the seventh day medication was stopped. About a week after medication was discontinued the WBC were 14,750 and three days later the temperature rose above 100°, being accompanied by pain in the small joints. At this time the magnesium preparation 75 grams (50 gm) daily was used instead of salicylates. The sedimentation time was 50 per cent. Following the administration of the drug subjective improvement was noted almost immediately. The temperature oscillated between 98.2° and 100.6° for eighteen days in spite of the medication. The leucocyte count was dropping, being 11,300 on April 7 and the sedimentation test was 25 per cent at about the same time. The amount of the drug was then increased to 90 grams (60 gm) daily, causing a drop in temperature to normal and after ten days of normal temperature a leucocyte count of 9,900 and a sedimentation time of 11 per cent, the patient was allowed out of bed and discharged a few days later. No nausea, ringing in the ears, jaundice, or urticaria were noted and the icteric index on discharge was 5 units. The patient had ingested 1,425 grams (950 gm) of magnephen.

CASE 3—(T V) A white man fifty years old, a painter, was admitted on May 2, 1931, because of fever and pain and swelling in the wrists, ankles elbow joints, and knees. No redness was noted by the patient. These symptoms were present for a short time before admission. He stated that during the past twenty years he had had attacks of "rheumatism" and three years ago had had a tonsillectomy. Venereal infection was denied.

The admission temperature was 101°. The patient, who was well nourished, was in pain and could not move his hands or feet. The teeth were in poor condition, and small tonsillar tags were present. The heart and lungs revealed no abnormalities, blood pressure 114/72. There were swelling and tenderness about the right wrist, elbow, and left ankle. No abnormalities were noted in the red blood cells by count or morphology on four studies. The admission WBC were 6800 and at no time was there a rise noted. The sedimentation time was 45 per cent on admission, the respective weekly determinations subsequently were 18 per cent, 10 per cent and 4 per cent. The blood Wassermann was negative and the icteric index 4 units both at the beginning and at the end of the treatment. This patient was given 75 grams (50 gm) magnesium pluvleinchoninic acid preparation daily, in 15 gram doses. As his pains were quite severe he was given codeine 1 gr every four hours for the first twelve

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CASE 2—(No 15611) An Italian boy thirteen years old was admitted on February 27, 1931, complaining of pains in the knees, ankles, wrists, and small joints of the hands for ten days. The present attack was initiated by a sore throat and cold in the head. There was a history of a previous attack of rheumatic fever three years before and the patient was under observation in the out patient department. Other than a systolic murmur on his former admission, no abnormalities of the heart were recorded. He had had measles in early childhood and a tonsillectomy when three years old. The physical examination revealed a well nourished and well developed pale boy. The previously observed systolic blow at the cardiac apex was noted, the liver was just palpable and there were swelling and tenderness in both ankles and the right third metacarpophalangeal joint. On admission white count was 12,500 with 72 per cent polys. The Wassermann was negative and the urinalysis revealed a trace of albumin. He was treated with sodium salicylate, 90 grains daily as his temperature was 104° and he was obviously in the acute phase of the disease. We preferred salicylates at first here, since we were eager to bring about symptomatic relief rapidly and did not feel justified in experimenting with a drug about which we knew little. On the third day he was practically symptom free on the fifth day the temperature dropped to normal and on the seventh day medication was stopped. About a week after medication was discontinued the WBC were 14,750 and three days later the temperature rose above 100°, being accompanied by pain in the small joints. At this time the magnesium preparation 75 grains (5.0 gm) daily was used instead of salicylates. The sedimentation time was 50 per cent. Following the administration of the drug subjective improvement was noted almost immediately. The temperature oscillated between 98.2° and 100.6° for eighteen days in spite of the medication. The leucocyte count was dropping, being 11,300 on April 7, and the sedimentation test was 25 per cent at about the same time. The amount of the drug was then increased to 90 grains (6.0 gm) daily, causing a drop in temperature to normal and after ten days of normal temperature a leucocyte count of 9,900 and a sedimentation time of 11 per cent, the patient was allowed out of bed and discharged a few days later. No nausea, ringing in the ears, jaundice, or urticaria were noted and the icteric index on discharge was 5 units. The patient had ingested 1,425 grains (95.0 gm) of magnephen.

CASE 3—(T V) A white man fifty years old, a painter, was admitted on May 2, 1931, because of fever and pain and swelling in the wrists, ankles, elbow joints, and knees. No redness was noted by the patient. These symptoms were present for a short time before admission. He stated that during the past twenty years he had had attacks of "rheumatism" and three years ago had had a tonsillectomy. Venereal infection was denied.

The admission temperature was 101°. The patient who was well nourished, was in pain and could not move his hands or feet. The teeth were in poor condition, and small tonsillar tags were present. The heart and lungs revealed no abnormalities, blood pressure 114/72. There were swelling and tenderness about the right wrist, elbow, and left ankle. No abnormalities were noted in the red blood cells by count or morphology on four studies. The admission WBC were 6,800 and at no time was there a rise noted. The sedimentation time was 45 per cent on admission the respective weekly determinations subsequently were 18 per cent, 10 per cent and 4 per cent. The blood Wassermann was negative and the icteric index 4 units both at the beginning and at the end of the treatment. This patient was given 75 grains (5.0 gm) magnesium phenylacetic acid preparation daily, in 15 grain doses. As his pains were quite severe, he was given codeine 1 gr every four hours for the first twelve

hours Within twenty four hours the temperature became normal and remained so for the rest of his hospital stay The swelling and tenderness disappeared from the joints involved, by the third day He frequently complained, however, of pain in the ankles, knees, wrists, and elbows No swelling or tenderness accompanied these pains The magnephen was then increased to 90 grains (6.0 gm) on May 14, daily, with the consequent relief of pain The drug was discontinued on May 28, 1931 This patient received a total of 2,130 grains (142 gm) and at no time were toxic symptoms observed After he was free of symptoms tonsillar tags were removed and he was then discharged on June 15, 1931

CASE 4—(Hospt No 15810) On March 25, 1931, a thirty eight year old Italian was admitted because of pain in the right knee and shoulder He had been ill for three days and the above symptoms were accompanied by a sore throat, loss of appetite, and fever The physical examination revealed a well nourished male whose teeth were in poor condition, the throat congested, the tonsils small, red and cryptic The heart and lungs presented no abnormalities The right knee and shoulder joints were tender and hot There was considerable limitation of motion, but no swelling The red blood cells revealed no abnormalities The white count was 15,600 with a 73 per cent poly count The blood Wassermann was negative Blood sugar 80, nonprotein nitrogen 40 The icteric index was 4 and the sedimentation time 50 per cent The urine revealed nothing abnormal On admission in the acute phase with severe joint pains and a temperature of 103°, he was placed on 75 grains (5.0 gm) of magnephen in 15 grain doses Symptomatic relief was noted in three days and the temperature was normal in five The sedimentation time, however, was still high, 25 per cent The medication was, therefore, continued for eighteen days in spite of the normal temperature, until both the leucocyte count and sedimentation time became normal, 8,200 and 5 per cent, respectively The medication was then discontinued and he was allowed out of bed No toxic symptoms were noted at any time, even though the patient had taken 1,575 grains (105 gm) of the medication

SUMMARY AND CONCLUSIONS

From data of the case reports it is obvious that the magnesium cinchophen with the added magnesium oxide fulfills certain desiderata, namely, antipyresis, analgesia and freedom from toxic symptoms, even when administered in therapeutic doses The magnesium apparently intensifies the phenyleinchoninic acid effect, thus making smaller dosage adequate and consequently the medication need not be pushed to a point of toxicity before symptomatic relief is obtained It is also apparent that this preparation does not cause cessation of activity as the leucocytosis and elevated sedimentation time persist, in spite of the alleviation of symptoms We have given the magnesium phenyleinchoninic acid (magnephen) compound in large doses The first patient received 1,875 grains (125.0 gm) over a period of 25 days, the second case had 1,425 grains (95.0 gm), the third 2,130 grains (142.0 gm) over 26 days, and the fourth 1,575 grains (105.0 gm) over a period of about 25 days In none of these were there evidences of drug toxicity We feel that these results are sufficiently encouraging to warrant further careful study of the magnesium phenyleinchoninic acid compound which promises to do all that is claimed for salicylates and cinchophen in the treatment of rheumatic fever, without producing any disagreeable or toxic symptoms We have not studied the effect of this preparation on the kidney The urines of our patients on discharge revealed no abnormalities to cause further tests of renal function We have also kept in mind the reports of the occasional harmful effect of phenyleinchoninic acid preparations on the liver The patients were observed for evidences of jaundice and the icteric index was

done on admission and discharge. No evidence of liver injury was noted clinically, and the icteric index showed no change after the ingestion of considerable quantities of the drug.

From our clinical study of seven cases of rheumatic fever, four of which we have discussed in detail, we offer the following conclusions:

1 Magnesium oxide in combination with magnesium cinchophen (magnephen) is an effective antipyretic and analgesic in the treatment of rheumatic fever.

2 The therapeutic dose, because of the magnesium potentiation, is much smaller and does not produce toxic symptoms.

3 Large doses such as 2,130 grains (142 gm.) over twenty-six days were well tolerated.

4 This preparation does not influence the activity of the rheumatic virus as is evidenced by a persisting leucocytosis and an increased sedimentation time. It produces symptomatic relief by reducing the temperature and relieving the arthralgia.

5 The advantage of the preparation is the absence of toxicity, when administered in therapeutic amounts. This has been our experience with the cases studied.

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THE PREDIABETIC STATE ITS TREATMENT BY THE LOW CARBOHYDRATE DIET AND THE REDUCTION OF WEIGHT*

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MANY writers recognize in the obese an incipient impairment of carbohydrate metabolism which is known as the prediabetic state¹. The predisposition of the obese to diabetes mellitus is a generally accepted fact². Numerous cases of diabetes must have had their beginning in a state of obesity with a similar impairment of function. The question of proper prophylaxis for this group, against diabetes, logically arises.

This should consist, theoretically, of a reduction in weight and a low carbohydrate diet so that the metabolic apparatus may recover its function. The amount of body tissue to be maintained will thus be lessened and the ingested carbohydrate to be metabolized will be lowered. Whether or not this therapy is successful can best be determined by repeated tests of the carbohydrate tolerance.

The Brill test meal of 100 grams of carbohydrate, 26 grams of protein and 27 grams of fat is used as a test of the carbohydrate tolerance in this clinic^{3, 4, 5}. It is given as a breakfast in the fasting state. A blood sugar is taken by venipuncture immediately before and again two hours following the ingestion of the meal. The night urine is collected one hour before the test, while that for the four hours following makes up the second specimen. The blood sugar values are determined by the Benedict copper method, the normal reading of which is 70 to 90 mg. per 100 cc. of blood⁶. The test is considered normal if a blood sugar of 100 mg. or less is attained in the second blood specimen taken two hours after the meal.

In Table I ten cases of obesity with an impaired tolerance are presented, hence prediabetics, as determined by the above method. All cases have been thoroughly studied in this clinic and none recorded in which were found factors that were known to affect the test. The heights and weights were determined minus clothing and without shoes. From these, and a normal standard,⁷ the percentage overweight was calculated. The age given in each case is that at the first tolerance test. After a varying period on a restricted low carbohydrate diet during which weight was lost, the meal was repeated and the improved tolerance found as tabulated in each second test of Cases 1 through 10.

The rationale of the treatment is further justified by consideration of Cases 11 and 12 (Table I) both of which demonstrate the loss of tolerance for carbohydrate through lack of diet and increasing obesity.

It would appear that the carbohydrate tolerance of the prediabetic can be improved by the reduction of weight and proper diet and that it depreciates with

*From the Diabetic Service of Dr. F. R. Wright at the Clifton Springs Sanitarium and Clinic.

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gain in weight and dietary indiscretions. The relative importance of the low carbohydrate diet and the reduction of weight needs no discussion. Observance of the first, obviates the second and, to this writer, is paramount.

These facts should be brought to the attention of the obese and prediabetic. It is only by such prophylaxis that a large group may be saved from future diabetes mellitus.

TABLE I

CASE	DATE	SEX	AGE	HEIGHT		WEIGHT		BLOOD SUGAR MG 100 CC		% URINE SUGAR		DUT	
				FT	IN	LBS	% ABOVE OR BELOW NORMAL	FASTING	2 HR	FASTING	4 HR	C	P
1	12/28/27 12/27/30	F	55	5	3	178 168	+20 +13	118 85	115 100	0 0	0	102	75
2	10/16/27 3/21/31	F	50	5	5	170 115	+12 +3	97 70	100 77	0 0	0	1200 cal	
3	8/11/28 8/11/30	F	38	5	6	181 155	+20 0	88 75	107 91	0 0	0	108	75
4	10/11/26 9/12/28	M	56	5	7	181 171	+10 +1	89 82	132 110	0 0	0	126	90
5	9/25/28 5/ 2/29	F	27	5	0	131 115	+1 -9	96 75	170 100	0 0	0	135	90
6	9/15/29 10/ 3/29	F	37	5	1	118 110	+6 0	95 97	132 95	0 0	0	150	60
7	12/ 6/30 2/29/31	M	27	5	7	261 221	+72 +53	97 79	120 81	0 0	0	225	120
8	11/30/25 7/ 8/27	F	40	5	1	168 140	+11 -5	100 80	132 81	0 0	0	120	72
9	5/10/30 8/15/30	F	50	5	1	198 171	+32 +15	80 70	110 88	0 0	0	120	72
10	5/19/28 11/29/30	M	44	5	7	195 165	+22 +1	101 72	137 75	0 0	0	135	90
11	1/29/30 12/17/30	F	69	5	1	183 193	+17 +21	91 91	83 111	0 0	0	No restrictions	
12	10/18/28 9/17/29	M	45	5	6	190 210	+26 +35	100 95	97 116	0 0	0	No restrictions	

CONCLUSIONS

1 Repeated carbohydrate tolerance tests on the obese and prediabetic demonstrate that tolerance depreciates with increasing weight and without a low carbohydrate diet, but that it improves with loss of weight and a diet low in carbohydrate

2 It is suggested that the obese and prediabetic be warned of this danger and advised of the necessity of reduction of weight and a restricted carbohydrate intake

3 The development of diabetes mellitus in a large group might be prevented by these measures

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THE STERILIZATION AND STANDARDIZATION OF PAPAIN PREPARATIONS INTENDED FOR SURGICAL USE*

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PROTEOLYTIC enzymes are being used by Ochsner and Garside¹ and others as a surgical means of preventing the reformation of peritoneal and pericardial adhesions. According to this procedure adhesions are divided by surgical means, and their reformation prevented by the presence of weak protease solutions. The fibrinous exudate is presumably digested to such extent as to prevent the formation of fibrous tissue. In contrast to trypsin, papain preparations have proved much more suitable in that they are more consistently effective and, further, are effective at spectacularly low activity concentrations.¹ This is apparently due to the markedly greater stability of the vegetable enzyme, papain in aqueous solution and to its capacity to act uninhibited in the presence of serum. Quantitative comparisons of this sort and comparative rates of deterioration in the peritoneal cavity, as determined by methods described here, are reported in another communication.²

This paper describes the sterilization procedure, the storage behavior and the activity standards of papain preparations which are being used for experimental and clinical work.

1. PREPARATION OF A STERILE PRODUCT

In a previous report³ sterile and stable trypsin preparations were conveniently obtained by two procedures. These have been applied in the case of papain with varying success. The first, which consisted in pressure filtration of a glycerine extract of the powder through Berkefeld filters, was discarded because of the relative instability of the glycerine papain filtrate. The second procedure, which consisted in a similar filtration and subsequent precipitation of the active powder with alcohol and ether under sterile conditions, presented no unexpected difficulties when applied to papain and the stability of the resulting powders was found to be entirely adequate. The firm of Parke Davis and Company have cooperated in developing a product of this latter type intended for clinical use.

Glycerin Papain Filtrates—In the same way as with trypsin, glycerine extracts of papain filtered by pressure through Berkefeld filters (N) furnished products of uniform activity. Using 5 gm. of powder dissolved or suspended in 100 cc. of 60 per cent glycerine, the activity of the filtrate is such that about 22 cc. is equal to one gram of the dry powder.

Numerous assays on 12 different lots over periods up to four months showed that filtrates stored open in the refrigerator averaged a 50 per cent activity-loss.

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in one month, while ampouled filtrates stored in the refrigerator averaged a 50 per cent activity-loss in four months. The rate of deterioration was observed by two methods to be described in Section 2. One is based on the time required to disintegrate approximately equal amounts of glycerine-stored fibrin and the other is expressed as units of activity necessary to dissolve the gelatine on a strip of photographic film.

A typical filtrate stored open in the refrigerator showed initially a "fibrin digestion time" of one and eight-tenths hours, after six days, two and two-tenths hours, after fourteen days, three and one-half hours and after ninety days, eight hours. In each digestion test 0.5 c.c. of the filtrate was used. Using this amount of filtrate, the "fibrin digestion time" for several freshly prepared lots was consistently within the limits of one and one-half to two hours. As these latter tests ranged over a period of four months, it is considered that the digestibility of the glycerine stored fibrin was not much altered.

Using the photographic film method and expressing activity in arbitrarily defined units the same type of storage behavior was noted. Fresh preparations exhibited 45 to 48 activity units per cubic centimeter. Typical assays on filtrates stored open in the refrigerator showed the following unitages per cubic centimeter: nine days, 42 units, nineteen days, 31 units, thirty-five days, 24 units, one hundred and five days, 20 units.

A lesser number of observations (about 10) were obtained with filtrates stored in sealed glass ampoules. The average indicated a slower rate of deterioration than open preparations.

Although considerable variations were observed in the deterioration rate of various specimens, the results were sufficiently consistent to indicate the inadvisability of glycerin filtrates for general clinical use. Because of the convenience of preparation and use, however, this type of product has been useful in laboratory experimentation.

That the activity of trypsin in glycerin solutions is much more constant was indicated in the previous report and has since been supported by further observations. One ampouled specimen, for instance, stored in the refrigerator for eight months exhibited an activity-loss of less than 10 per cent.

No experimental explanation was developed for this lesser stability of papain on storage in glycerin although some unsuccessful attempts were made. Contact with mercury vapor for one month at ice chest temperatures indicated no particular inactivation from this source. Slow streams of oxygen and of carbon dioxide bubbled through the solutions for twelve and fifteen hours at ordinary temperatures did not cause an activity-loss greater than 10 per cent. When poured in shallow dishes and exposed to ultraviolet rays for two hours, the same order of stability was noted. The aqueous glycerin solutions at 60 per cent concentration were shown not to undergo dilution by absorbed moisture under the usual conditions of storage.

Filtered and Precipitated Papain Powders—According to this procedure, aqueous or 60 per cent glycerine solutions of papain were filtered as before, precipitated with 5 volumes ethyl alcohol, treated successively with anhydrous alcohol and anhydrous ether and allowed to dry in a desiccator containing, sep-

arately concentrated sulphuric acid and sticks of alkali. Separation of the powder from the suspension liquid is best effected by centrifuging. Ordinarily, 80 to 90 per cent of the filtrate activity can be recovered in the precipitate. Chlorotone or acriflavine can be used as precautionary antiseptics, since they have only a very slight effect on papain activity.

In that these operations required sterile technic throughout, the laboratory preparation was most conveniently effected by placing one to two c.c. quantities of filtrate in ordinary test tubes and carrying out the operations of precipitation, centrifuging and drying in the same tube in which it was ultimately sealed for use. This was possible since these quantities represented as much or more than the usual therapeutic dosage.

Preparations of this sort exhibited the normally expected stability as determined over periods up to forty-five days. One preparation stored for twenty days at 50° in a sealed ampoule exhibited an activity loss not greater than 10 per cent.

According to the method now used by Parke Davis and Company aqueous extracts are prepared by overnight contact, filtration and precipitation with methyl alcohol. Other details are much the same as described above. These products are more completely soluble than the untreated powders and the relation of protein-dissolving activity to protein-hydrolyzing activity is distinctly greater than with the untreated powders. These latter relations are given in Section 2. The protein-dissolving activity of these sterile powders now being used in the laboratory and in the clinic, corresponds roughly milligram for milligram, to that of the unsterilized stock preparations. This is a matter of convenience in expressing activity as most of the surgical and pharmacologic work done here has been based on either one or the other of these two products.*

With the expectation of simplifying the preparative procedure, observations were made as to the storage behavior of the precipitate when allowed to remain in contact with the precipitating alcohol. The deterioration rate was somewhat greater than that of the glycerin filtrates but of the same general order. One preparation, for instance, kept sealed in the ice chest for thirty-five days, showed a change of "fibrin digestion time" from three to six hours. These observations have an incidental interest in demonstrating that the papain precipitates are not markedly affected by the presence of the precipitating alcohol. Accordingly, time of contact with alcohol is not an important factor in standardizing the preparative procedure.

Sterilization by Carbon Disulphide—Recently, Vandeveld⁴ has reported effective sterilization of enzyme powders by prolonged treatment with freshly distilled CS₂ at ordinary temperatures. Periods of several days were considered adequate, although in an earlier report⁵ six weeks of contact were employed in sterilizing flours. No statements are given as to the number and types of bacterial contaminants. From the following observations it is seen that the method, as described, is not suitable for the sterilization of papain powders when highly contaminated with *B. subtilis*.

*The activity and solubility of these untreated stock preparations did not differ essentially from a stock preparation of Merck's White Label papain. However a special papain preparation kindly supplied by Wallerstein laboratories exhibited a substantially greater activity.

Portions (100 mgm) of untreated powder from the same lot throughout were weighed into sterile test tubes and covered with about 6 c c of CS₂. The tubes, already drawn out, were sealed by flame. After standing for various periods of time, the tubes were broken and the CS₂ allowed to evaporate under the protection of sterile cotton plugs. The powders were dissolved in sterile water and 1/10 of the solution cultured on agar plates. The results are given in Table I.

TABLE I

TIME OF CONTACT	CARBON DISULPHIDE	COLONIES PER 100 MG	
		NEUTRAL AGAR	MEAT DIGEST AGAR AT PH 7.6
—	Blank	1300	
5 days	Ordinary CS	2900	
60 days	Ordinary CS	450	350
30 days	Freshly distilled	620	
45 days	Freshly distilled	360	100
45 days	Freshly distilled	350	270

Once when this procedure was modified by the addition of intermittent heat exposure, complete sterilization was obtained. During the first week of a thirty-day storage period the sealed tube, containing the powder under freshly distilled CS₂, was kept at a temperature of 56° for a total of thirty-five hours in five intermittent periods. The effect of this latter treatment on proteolytic activity was not ascertained, although it was shown that five- to six-day contacts did not diminish activity.

2. DEFINITION OF STANDARD ACTIVITY

Drug houses have assayed papain preparations by a method involving the digestion of raw meat. It was considered desirable to add another definition of activity based on a more uniformly reproducible substrate and based on a definite chemical change. For this purpose "Coignet's Silver Label Gelatine" was used and the extent of proteolysis ascertained by formal titrations before and after digestion.

Two further methods of assay do not furnish as reproducible definitions of activity since they were more directly intended as methods adapted to the other phases of these studies. The first, the determination of "fibrin digestion time," provides only an approximate estimation of activity, but is relatively simple and convenient. The second, termed the photographic film method, is more exact and gives results in direct units of activity, but must be referred to the activity of some standard powder.

TABLE II

MEDIUM	PHOTOGRAPHIC FILM UNITS PER GRAM OF PAPAIN POWDER
Distilled water	220
Sodium citrate solution (1%)	1,000
HCN solution (0.15%)	1,850
H ₂ S solution (0.15%)	8,100

In each of these methods, sodium citrate was used since it is one of the more convenient of the so-called "activators." The relative influence of such substances is indicated by figures shown in Table II.

With fibrin digestions the same order of relations is obtained but the differences are not so marked.

Commercial Assay—The method employed by Parke Davis and Company in standardizing their papain preparations is as follows, according to their description. Ten grams of shredded lean raw beef is mixed with 0.325 gm of papain and 50 cc of water. Digestion is carried out for six hours at 52-55°, the bottle being shaken gently for one minute every fifteen minutes. At the end of digestion the contents are poured into graduated tubes and allowed to settle. With standard, market preparations the undigested residue measures about 7 cc.

Formol Titrations of Gelatine Digestions—Five per cent solutions of air-dried gelatine containing 13.5 per cent moisture (determined by drying to constant weight at 110°) were prepared by heating at 37.5° and were always used immediately. Twenty cc of this solution was mixed with 18 cc of a 5 per cent solution of sodium citrate crystals (U.S.P.) and 2 cc of N/10 NaOH and the mixture brought to a temperature of 37.5° in a thermostat, whereupon 20 cc of a 2.0 per cent unfiltered suspension or solution of the enzyme preparation was added. After digestion for ninety minutes, 50 cc of the mixture was withdrawn and added to 50 cc of 37 per cent formaldehyde solution not quite neutralized to phenolphthalein. Using phenolphthalein as an outside indicator, this mixture was titrated with N/10 alkali and the titration of a blank subtracted. Blanks were obtained by carrying out the same incubation with boiled enzyme preparations. The activity of stock preparations of Fairchild's trypsin and of Parke Davis' untreated papain is represented by a titration change of 10.80 cc and 3.70 cc N/10 alkali, respectively. The activity of two lots of Parke Davis' sterilized papain was represented by a titration change of 2.30 cc N/10 alkali. Moisture content of the untreated trypsin and papain was 7.2 per cent and 5.9 per cent, respectively, as determined by drying to constant weight at 110°.

Determination of "Fibrin Digestion Time"—Glycerine stored fibrin of beef blood, in amounts corresponding to 40 or 50 mg when oven-dried was washed free of glycerine, shredded with a scalpel and the moist fibrin introduced into 10 cc of digestion mixture. Digestions in duplicate or triplicate were carried out in a 1.0 per cent solution of sodium citrate crystals (U.S.P.) at a temperature of 40°. The time for an estimated disintegration of 70 per cent of the shreds is taken as the "fibrin digestion time." After disintegration is once evident, complete disintegration follows rapidly. Disintegration without complete solution is the usual course with papain digestions at this temperature. At higher temperatures, 70°, for instance there is no residue of disintegrated shred. The standard preparations showed the following "fibrin digestion times": 1-250 Parke Davis' papain (sterilized) 1.8 hr, 1-250 Parke Davis' papain (untreated), 2.0 hr, 1-1,000 Fairchild's trypsin 0.6 hr.

Photographic Film Method—The procedures described by Gates and by Gilman and Cowgill have been adapted to the requirements of these studies. The method has the advantage of being relatively quantitative and rapid at very

low activity concentrations and is uniquely suited to the determinations of proteolytic activity at concentrations (1-15,000 papain) which approach the conditions of clinical use.² Mechanical features were essentially the same as used by these authors except that cheap alloy wedding rings of 19 mm inside diameter were used in place of copper wire rings. Eastman Dupli-tized Superspeed X-ray films, sensitized by fifteen seconds exposure to a 15 watt Mazda light bulb at a distance of 26 inches, were developed for two minutes in Eastman X-ray developer and immersed in the fixing solution for four minutes. The gelatine emulsion was stripped from one side of the film with warm water, precautions being taken to leave the other side unaffected. As the films were of 14 by 17 inch dimensions, they furnished a considerable number of single strips of reasonably uniform resistance. Digestions were carried out in a thermostatically regulated water-bath controlled to 0.05°.

Results were arbitrarily expressed on the basis of a definite activity concentration, i. e., that concentration which will convert the photographic film to one-half of its original photometric reading upon digestion for forty minutes at 27.50° in a solution of 1 per cent sodium citrate crystals (U. S. P.). This concentration being taken as unity, the unitage per gram of powder is simply the dilution to which the powder must be carried before its solution reaches this point of activity. The activity of Parke Davis' papain (untreated), which serves as the standard of reference, is set at 1,000 units per gram (anhydrous). Accordingly, one gram when diluted to 1,000 cc represents a solution of exactly the activity necessary to convert the photometric reading to one-half the original reading. The resistance of various films will diverge considerably from this arbitrary standard and must be calibrated with each series of determinations.

In the study by Gilman and Cowgill with pepsin the photometric change was directly proportional to the logarithm of enzyme concentration. In these studies, the same type of monomolecular reaction was approximated with trypsin but not with papain. With papain, the concentration range between total digestion and zero digestion was very much sharper. This necessitated a greater number of trial assays but, correspondingly, increased the order of accuracy.

The unitage of the standard powders by this method is as follows: Parke Davis' papain (untreated), 1,000; Parke Davis' papain (sterilized), 1,100; Fairchild's trypsin, 24,000.

The Casein Digestion Test—The U. S. P. or Fuld Gross method is not suited to papain determinations because of the insoluble products formed by the action on casein.

SUMMARY

Filtered glycerin extracts of papain evidence sufficient instability to preclude their clinical use. Filtered and precipitated powders constitute a product suitable for the purposes described. The activity of such powders is defined according to three procedures. Prolonged treatment with CS₂, as described by Vandeveld, is not adequate for the sterilization of heavily infected papain powders.

This work has been carried out in conjunction with the Department of Surgery and I wish to acknowledge the various types of assistance which they have rendered.

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LABORATORY METHODS

NOTE ON THE CALCULATION OF URINE SOLIDS*

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THE amount of solid matter dissolved in the twenty-four-hour urine elimination is a rough measure of the nutritional level. The actual determination, a necessity in certain types of metabolic study, is attended by no little difficulty if error is to be reduced to inconsiderable proportions. Evaporation *in vacuo* at room temperature is time consuming, and the last traces of water are difficult to remove. The removal of water by heating, on the other hand, leads to chemical decomposition of the nitrogen-containing substances—primarily urea—with a loss of ammonia and carbon dioxide for which allowance must be made by additional analyses. Neither procedure is adapted to clinical use and for many years the estimation of the specific gravity correlated with the volume has been the usual simple expedient adopted.

The specific gravity of a complex solution, such as is the urine, is a summation of the relative amounts and densities of the various dissolved substances. Further, in dilute solutions the relationship of concentration to specific gravity approaches a linear function. From this fact, if the composition of the solution were fairly constant in the relative proportions of the several constituents, a fairly constant ratio should exist between the specific gravity and the actual amount of dissolved matter. An early recognition of this probability led to investigation, and several decades ago a coefficient was determined which is known usually as Haeser's, Roberts', etc., all resting on the basis of the simple arithmetical relationship—

$$K = \frac{\text{Total Solids per liter}}{1000 (\text{Specific Gravity} - 1.000)}$$

For measurements at 15.5° C, the earlier standard temperature, "K" was found to approximate the value of 2.33. Nearly thirty years ago, Long¹ revised this value by an experimental study and offered the ratio, $K = 2.6$ when specific gravities are determined at 25° C. A simple computation demonstrates that the two coefficients are in complete numerical agreement, the larger value of Long compensating for the lower specific gravity produced by thermal expansion of the solution. Both Long and his predecessors selected so called "normal" urines for their investigations, a fact which led to an artefactual constancy in their experimental results. The two chief constituents of the urine in point of amount are urea and sodium chloride. Weight for weight, at concentrations found in urine, the salt influences the specific gravity between two and three

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times as much as does the urea. For example a 2 per cent solution of the latter has a specific gravity of 1.0058, of the former 1.0146. Since together these two substances constitute from 50 per cent to 80 per cent of the total dissolved matter, they conjointly exercise the principal influence in determining the density, as their several influences are so divergent, due cognizance must be taken of their ratio. In the "normal" urine this assumes a value of approximately two parts of urea to one of sodium chloride.

To reduce these influences to arithmetical terms, the writer has made a series of measurements of densities of a group of solutions containing the two substances in varying proportions.

The desired amounts of urea, sodium chloride, and water were weighed, on balances sensible to 1 centigram, into stoppered Erlenmeyer flasks of 1 liter capacity, the final weight of the solution being 500 grams. After complete solution and uniform concentration had been secured by prolonged shaking exactly 250 grams of this solution was diluted with an equal weight of water. After thorough mixing, the operation was repeated to produce a more dilute solution and that in turn gave a fourth. Recognizing that an initial error would vitiate an entire series, a duplicate of some one of the dilute solutions in each series was supplementarily made by careful weighing and its specific gravity was compared with that of the original solution made by dilution. These checks in every instance justified the confidence placed in the original observations. The urea was of "highest purity" quality, the sodium chloride of equally high grade and yielded on analysis less than 0.1 per cent of moisture. The solutions were made in distilled water.

As soon as prepared the solutions were placed in dry, clean, glass-stoppered bottles and these stored in a cool room. The densities were determined inside of a few hours, using a carefully standardized Westphal balance. These balances are commonly adjusted to give then 0 reading at 15.5° and as the majority of urinometers on the market are constructed for this temperature, it was taken as the standard. As the weather was very warm, some little difficulty was experienced at first in holding the solutions at the proper temperature. The substitution of a silvered Dewar cylinder of some 200 milliliters' capacity for the usual glass receptacle, made it easily possible to maintain constant thermal conditions. A carefully standardized thermometer gave the temperature of the solution, the use of small glass stirrers ensured thermal homogeneity. The initial results are collected in tabular form (Table I).

A number of the higher concentrations fall outside the range of physiologic probability and are included solely to define the trends of the curves. The values in brackets at the low concentrations are extrapolated, a warrantable procedure as the relationships are known to be substantially rectilinear at these levels.

A review of the data shows some interesting features. The "K" values approximate constancy for any given ratio, within the usual concentration limits, the variation is of the order of the ordinary observation error. The coefficient of the solution with the ratio of the "normal" urine, i.e., 2 to 1, is the figure of the earlier experimentally determined constant. Seemingly at these concentrations the algebraic summation of the other urinary constituents produces a solution of a density approximating that of the urea salt mixture. That this would hold

TABLE I
SPECIFIC GRAVITIES AT 15.5°C AND "K," VALUES FOR SOLUTIONS OF UREA AND SODIUM CHLORIDE

UREA %	UREA	UREA 12 =— N ₂ Cl 1	8 =— 1	6 =— 1	4 =— 1	3 =— 1	2 =— 1	1 =— 1	0.5 =— 1	0.25 =— 1	N ₂ Cl FQUVA FNT %
16	10162 3 62	—	—	—	1 0712 2 90	—	—	—	—	—	1 1190 1 50
8	1 0232 3 53	1 0267 3 33	1 0294 3 15	1 0315 3 05	1 0366 2 83	1 0428 2 60	1 0518 2 43	1 0808 2 14	1 1420 1 93	—	1 0385 1 45
4	1 0115 3 52	1 0132 3 33	1 0146 3 13	1 0157 3 02	1 0184 2 77	1 0211 2 58	1 0257 2 39	1 0402 2 07	1 0609 1 84	1 1321 1 71	1 0291 1 41
2	1 0058 3 47	1 0067 3 26	1 0074 3 08	1 0078 3 01	1 0093 2 71	1 0105 2 57	1 0130 2 34	1 0199 2 05	1 0351 1 77	1 0650 1 61	1 0146 1 39
1	1 0029 3 45	1 0034 3 20	1 0037 3 05	1 0039 3 00	1 0046 2 73	1 0053 2 53	1 0065 2 32	1 0099 2 05	1 0175 1 74	1 0325 1 59	1 0073 1 38
0.5	(1 0014) 3 45	(1 0017) 3 19	(1 0018) 3 05	(1 0019) 3 00	1 0024 2 61	1 0027 2 48	1 0033 2 30	1 0049 2 05	1 0087 1 74	1 0162 1 57	1 0037 1 36
0.25	(1 0007) 3 45	(1 0008) 3 19	(1 0009) 3 02	(1 0009) 2 99	1 0012 2 60	(1 0013) 2 47	(1 0016) 2 28	(1 0021) 2 05	1 0043 1 75	1 0081 1 56	(1 0018) 1 35

in urines of markedly abnormal composition is doubtful, but such urines are exceptional

On the other hand, as was to be anticipated, the "K" value varies over a very wide range as the ratio of the two substances changes. A pure 2 per cent urea solution has a "K" value of 3.47, while the "K" of the equivalent salt solution is but 1.39.

The amount of urea eliminated is directly influenced by the level of protein catabolism, as was shown most graphically by Polin² many years ago and since confirmed by a host of investigators. A variety of toxic states increase the urea output, while lowered renal permeability will depress its elimination. The possibility of urea storage is a factor that cannot be discussed here. Sodium chloride in largest measure reflects the level of salt intake. In fasting³ it sinks to very low levels as well as in a variety of pathologic conditions of which the exudative phase of pneumonia is a striking example.

As the variation with the concentration of the "K" value for any given ratio has already been shown to be of but minor proportions it is a simple matter to plot the curve of average "K" values over a wide range of ratios. For the sake of convenience these may be reduced to tabular form, using round numbers only in recognition of the very approximate character of the data.

TABLE II

"K" VALUES FOR VARIOUS RATIOS OF UREA AND SODIUM CHLORIDE						
Urea	100	50	25	12	10	8
=	—	—	—	—	—	—
NaCl						
K	= 3.5	3.4	3.3 ⁵	3.2 ⁵	3.2	3.1
	6	4	3	2	1	0.5
	0.25					
	3.0	2.7	2.5	2.3	2.0	1.8
						1.6

To calculate the total solids per liter, the following formula is derived from that first given

Total Solids = 1000 K (Specific Gravity—1.000) per liter. Substitution of the actual volume in c.c. for the 1000 gives a first approximation of the solid elimination in the specimen. To apply, determine the urea and sodium chloride by any of the standard quantitative procedures and use the "K" value indicated by their ratio.

Two points of exception should be noted in applying the formula. The presence of glucose in appreciable amounts necessitates a correction. This can most easily be applied by deducting from the observed specific gravity an amount

TABLE III

APPROXIMATE CORRECTION FOR SPECIFIC GRAVITY OF GLUCOSE SOLUTIONS							
15.5°				15.5°			
% Glucose	1	2	3	4	5	7.5	10
Correction	-.004	-.008	-.012	-.016	-.020	-.030	-.040

equivalent to that produced by the amount of sugar present Long¹ suggested this expedient for the sodium chloride, using a modified coefficient for the remaining solids

A second source of error is the so-called "ketone" bodies with acetone as the most disturbing factor The relative amounts of the several substances and the degree of salt formation of the two acids would be very variable and preclude the application of a simple correction such as is appropriate in the case of sugar

With these two exceptions, the use of the coefficients from Table II permits of a rough estimate of the dissolved matter of the urine on a parity in accuracy with the measurement of the specific gravity

Even so rough an estimate has a certain value for clinical interpretation and patently offers a definite advantage over the time honored practice

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A NONGLUCOSE REDUCTION PRESENT IN NORMAL AND INCREASED IN NEPHRITIC BLOOD*

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INCREASE of the blood sugar in cases of nephritis, first reported by Myers¹ in 1916, has been repeatedly confirmed, but by the use of improved methods, we now recognize a lower normal for blood sugar, and recognize too that the methods in general use clinically give glucose figures that are still too high as they include another reduction than that of glucose. However since the nonglucose reduction is small and practically constant in amount, clinical interpretation is not affected.

The true glucose content of the blood may be distinguished from the apparent glucose measured by oxidation methods by the loss in reduction after fermentation by yeast. Somogyi,² Fontes and Thivolle,³ and others have shown that yeast completely removes glucose from the blood within a few seconds and at ordinary temperatures, and that there is no other action of the yeast on the blood affecting reduction, as the same results may be obtained by allowing the yeast to act on the neutralized deproteinized filtrate. Somogyi and Kramer⁴ found the true sugar the same with various methods of estimation, while the nonglucose reducing body varied with different oxidizing agents.

The nonsugar reducing substance, estimated as glucose, was found by Somogyi and Kramer to be quite uniform (23 to 31 mg.), averaging 27 mg. using tungstate precipitation and the Shaffer-Hartmann method. With the Folin-Wu method, West, Scharles, and Peterson⁵ found the nonglucose reducing body 20 mg., and Benedict with the Benedict copper reagent found 117 mg. average. Bigwood and Wuillot⁶ found 14 to 25 mg. nonglucose reducing substance, using the Hagedorn and Jensen method (Table I). Controlled by fer-

TABLE I
REDUCTION IN BLOOD FILTRATES AFTER REMOVAL OF GLUCOSE BY YEAST "GLUCIDE X"

Somogyi and Kramer ⁴	av. 27 mg.	Shaffer-Hartmann
West, Scharles, Peterson	av. 20 mg.	Folin-Wu
Benedict ⁷	av. 12 mg.	Benedict copper method
Benedict ⁸	av. 22 mg.	Folin-Wu
Bigwood and Wuillot ⁶	av. 19 mg.	Hagedorn-Jensen
Folin and Svedberg (diabetics)	av. 20 mg.	Folin-Wu
Folin and Svedberg (diabetics)	av. 9 mg.	Folin
Present report	av. 21 mg.	Folin-Wu

mentation it has been shown that the nonglucose reducing body is precipitated by mercury,³ the filtrate containing only glucose as reducing substance. Thus the protein precipitant as well as the oxidizing agent are essential factors in the amount of reduction found in the blood and ascribed to the included glucose.

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Fontès and Thivolle give as rules for the control of a method for the estimation of blood glucose (1) to remove the glucose only (by yeast), (2) precipitate the proteins, (3) find no glucose in the filtrate, (4) on adding glucose to the filtrate to find it quantitatively. Their method they state fulfills these rules. Bigwood and Wuillot conclude "there is in the plasma and in whole blood reducing substances, probably glucidic, which yeast does not attack. These substances do not include uric acid or creatinine" except with very high nitrogen retention. Fontès and Thivolle think normal blood contains besides free glucose a non-glucose reducing substance which may be a "glucidic ether," provisionally called "glucide X," which is precipitable by mercury. The ether element is hypothetical, but the glucid nature they assume because of (1) its destruction by yeast, (2) the increase of glucide X along with free glucose by the action of adrenalin, (3) the decrease of glucide X with the use of insulin.

We have then, normally, about 20 mg. of reducing substance, glucide X, which is oxidized and estimated as, and with glucose by the Folin-Wu method in the tungstate filtrate, the various methods of estimation corresponding in this finding within the limits of error. Glucide X has been found a nonfermentable remainder in the tungstate filtrate after the destruction of the free glucose by yeast. The reduction, expressed as glucose, shows a variation in sensitivity to glucide X by the reagents of the different methods. Zinc and mercuric precipitants precipitate, or change, glucide X, leaving only the free glucose as reducing body and checking against the yeast method. The copper reagent of Fontès and Thivolle is so modified that it is not sensitive to glucide X after the action of yeast, although this body can be shown in the tungstate filtrate they use, after yeast fermentation, by other methods of oxidation. They measure glucide X by the difference in reduction between the tungstate and zinc filtrates. Glucide X is changed then by yeast, but not destroyed by it, and Fontès and Thivolle's method which is insensitive to this body after yeast, thus shows this interesting change in this reducing body, although they consider it fermented and destroyed by yeast, contrary to the finding of most writers. Our results were nearly all in accord with the findings of others, that glucide X reduces the Folin-Wu copper solution after the removal of glucose by yeast, and we estimated it thus. However we had some irregular results which are perhaps explained by the occasional modification of glucide X by yeast, preventing its action on the Folin-Wu reagents, a modification which regularly prevents its reduction of the copper solution of Fontès and Thivolle. Table II shows that this body cannot be demonstrated in some bloods after fermentation.

The difference between the Benedict modification of the Lewis Benedict method⁸ and the Folin-Wu, and the reducing body or bodies in column 3, Table II, is the subject of the present report. In the course of the laboratory examination of a case of nephritis, we found a considerable discrepancy between the blood sugar estimations by these two methods. The patient was able to be at his office most of the time, although constantly showing large amounts of albumin with a sediment of granular and cell casts in his urine, his blood urea N varied from 25 to 36 mg. per 100 cc. At the higher figure he had vertigo and headaches and was compelled to rest. His blood sugar by the Folin-Wu method had been about 100 mg. The last time we saw him his blood urea N was 31, sugar (F-W) 98 mg.,

TABLE II*
GLUCIDE X SOMETIMES FERMENTABLE

CASE	F W†	L-B	REDUC- TION	AFTER FERMENTATION BY YEAST		
				TIME	F W	L-B
Hypertrophied prostate	244	241	0	30 min	0	0
Glomerulonephritis	98	173	75	30 min	0	79
Chronic nephritis	100	137	37	30 min	0	40
Early interstitial nephritis	97	120	23	1 min hour	30 28	— —
Same, week later	100	144	44	1 min	22	80
				20 min	0	80
				hour	0	65
				day	0	70
Nephritis, hypertrophied prostate	144	200	64	1 min hour	30 0	
Interstitial nephritis	125	190	65	1 min	29	
				hour	30	
				3 hr	29	
				18 hr	18	
Interstitial nephritis	95	230	135	1 min	0	
Interstitial nephritis	150	240	90	1 min	0	

*Standard methods in standard quantities were used throughout this work (no micro-methods). The Benedict modification as in Underhill (ref 3). Baker chemicals. At least three fourths of the figures in the tables are the average of duplicate tests run by different individuals.

†F-W = Folin-Wu blood sugar method. L-B = Benedict modification of the Lewis-Benedict method. All figures milligrams per 100 c.c. of blood.

and 173 by the Benedict modification, showing a reducing body of 75 mg estimated as glucose, a difference far beyond any error inherent in the methods. The reducing body was not glucose, it did not affect the Folin-Wu copper reagent, and when the blood glucose was removed by yeast (thirty minutes) the Folin-Wu gave a zero reading while the picrate method gave a reading practically equal to the previous difference. (This later proved to be an exceptional result.)

It has been shown⁹ that the Benedict modification averages about 10 per cent higher than the Folin-Wu with dextrose solutions. With blood, the higher readings of the Benedict modification have caused it to be dropped and perhaps have somewhat discredited the simple and reliable picrate method of Myers which we have found checks so accurately with the Folin-Wu, that we have used it with the latter as a check on the Benedict modification for the estimation of the reduction which thus evidently is not due to the picrate alone. It was suggested by Professor Myers (personal communication) that the higher reading might be due to an increased sensitivity to "creatinine" of the Benedict modification over the original Benedict picrate method as modified by Myers and Bailey. The latter gives a creatinine reduction equal to that of an equal amount of glucose, Myers has proved and therefore negligible in any but exceptional bloods. On his sug-

gestion we therefore ran controls with the Benedict modification on glucose solutions to which creatinine was added, 1, 5, and 10 mg creatinine, with 100 mg glucose gave 105, 108, and 107 average readings

Table III gives the "y-reduction" in 25 normal bloods, taken after an eighteen-hour carbohydrate fast from students of athletic type at San Diego

TABLE III
Y REDUCTION IN NORMAL BLOODS

STUDENTS	L-B	F W	Y REDUC TION	STUDENTS	L-B	F W	Y REDUC TION
Br	149	82	67	Mg	114	86	28
Su	146	102	44	Wi	139	71	68
Wa	136	82	54	Wo	151	83	68
Ra	134	87	47	Ja	138	85	53
Ro	131	86	45	To	129	69	60
To	107	82	15	Jo	113	79	34
Je	114	86	28	Ph	130	78	52
Ma	114	83	31	OF	118	77	41
Mg	106	83	23	Co	124	79	45
Ma	103	76	27	Ar	120	80	40
Ro	89	75	14				
Bo	129	92	37				
Hu	97	80	17				
Ke	119	71	48				
Ma	104	80	24				

State College The y-reduction varies considerably in normal blood, from 14 to 68 mg, average 40.4 mg. Note that by the Benedict modification several of the normal students would have been considered as having glycemia. Table IV gives the y-reduction in the blood of patients with nephritis as evidenced by finding albumin and casts. Table V lists the negative findings. Among the nephritics of Table IV, 9 of the 41 examinations gave glucose over 120 mg by the Folin-Wu method, confirming previous reports of high blood sugar in nephritis, the picrate method shows 31 over 130 mg. Glycosuria was occasional, in only one of these cases.

The y-reduction in 7 tests on 4 patients with severe nephritis, heading Table IV, averages 108.7 mg, the average y-reduction of the other bloods in Table IV is 48.7 mg, and the average of all samples is 58 mg, much over the average of the normal bloods (40.4 mg). That this increase is not due to the creatinine bodies is shown by comparison with the creatinine findings, Table VI. The y-reduction is not necessarily due to one substance, and that this is possibly true of nephritic blood is suggested by the abnormal ratio of urea nitrogen to the non-protein nitrogen in 7 estimations, 6 cases, heading Table IV, the average urea nitrogen is 35 mg, nonprotein nitrogen 110.9 mg, ratio 1:3.2. It was our intention to learn the clinical significance of the higher reduction in nephritis by interpolating this finding in the "staircase" table of V. C. Myers, adding to the creatinine, uric acid and urea which formed his table, lowered phthalein output and fixation of specific gravity. We soon found this program too ambitious in a city without the facilities of a teaching hospital.

TABLE IV*

CASES AND DIAGNOSTIC DATA	DAYS	BLOOD CHEMISTRY	REDUCING SUBSTANCES		
			F W	L B	% REDUC- TION
Chronic nephritis, no albuminuria, few granular casts, fixed Sp Gr Occasional glycosuria (Dr St Sure "R")		urea N 23 NPN 77	109	135	26
Chronic nephritis, albumin 4+, hyaline and granular casts 4+ (Dr Stigall "C")		urea N 12 NPN 95 creat 1.3	100	293	193
Same, diet with reduced protein, 2 weeks		urea N 16 NPN 105	105	150	45
Same, third week		urea N 24 NPN 53	136	200	64
Uremia, coma, albumin 4+, casts 4+, moribund (Dr Barclay "W")		urea N 140 NPN 340 creat 6	107	224	117
General arteriosclerosis, chronic nephritis ascites P.S.P 11 per cent (Dr Potter "Br")	Feb	urea N 64.80 creat 3.35			
	Apr	urea N 60	192	260	68
	May		179	227	48
Trace albumin, no casts	Oct	urea N 37 creat 1.4 ur ac 3.2			
Died in December					
Arteriosclerosis, hypertension, cerebral hemorrhage, death (Dr Newmann "M")		urea N 20 NPN 75 creat 1.8	154	380	226
Hypertrophic prostate, retention, albumin and granular casts P.S.P 60 per cent (Dr Hall "Sw")	1	urea N 25	125		
	30		95	135	40
	48	urea N 23 creat 1.6	122	202	80
	55	urea N 25 creat 2.5	105	123	18
	60	urea N 15	85	149	64
	67	urea N 15	97	190	93
Hypertension, myocarditis, albumin and casts (Dr Hall, Naval Hospital "Co")	1	urea N 32	96	122	27
	3		85	124	39
	20		100	123	23
Hypertrophy of prostate, obstruction, cystitis proctitis (Dr Hall "Fw")	1	urea N 32 creat 2.5	75		
	7	urea N 28 creat 1.7	72	134	62
	14	urea N 32 creat 2.5	91	149	57
Chronic nephritis, myocarditis, recovering from hemiplegia, albumin granular casts (Dr Hall "Kr")	1	urea N 28 creat 1.7	81	127	46
	3		164	200	136

TABLE IV (Continued)

CASES AND DIAGNOSTIC DATA	DAYS	BLOOD CHEMISTRY	REDUCING SUBSTANCES		
			F W	L B	% REDUC TION
	13		118	181	63
	22	urea N 25 creat 2	87	139	52
	40	urea N 12 creat 17	76	145	69
Arteriosclerosis, hypertension hypostatic pneumonia, death Age 68 (Dr Stevenson 31170)		urea N 7	94	156	62
Persistent secondary anemia, low fixed Sp Gr (Dr Kennell "CR")			100	144	44
Arteriosclerosis syphilis, chronic nephritis myocarditis, albumin, hyaline and granular casts Age 63 (Dr Stevenson 28833)			110	127	17
Bronchopneumonia, lung abscess, age 78, albumin, granular casts (Dr Stevenson 28676)		urea N 11 creat 17 PSP 46%	104	133	29
Hypertrophy of prostate trace albumin, age 55 (Dr Stevenson 28757)		urea N 15 creat 17 PSP 37%	111	139	28
Salpingitis, albumin, granular casts, age 32 (Dr Stevenson 28487)			93	187	94
Syphilis, meningitis, albumin hyaline, granular casts, age 64 (Dr Stevenson 28841)		urea N 30 creat 2	82	119	37
Cancer breast, trace albumin, casts, age 67 (Dr Stevenson 28251)			110	139	20
Cellulitis hand, syphilis, trace albumin, casts, age 59 (Dr Stevenson 27901)			86	178	92
Endometritis, albumin, many hyaline, few granular casts, age 29 (Dr Stevenson 28500)			114	134	20
Luetic aortitis, trace albumin, casts, age 67 (Dr Stevenson 28552)		urea N 10 NPN 31 creat 17	104	123	19
Sinus operation, hemorrhages, convulsions (Mercy Hosp 438)		NPN 136 creat 32	130	167	37
Mastoiditis, age 42, trace albumin (22687)			71	114	43
Acute cholecystitis, age 54, albumin, granular casts (28944)			97	130	33
Arthritis, age 35, trace albumin hyaline casts (28829)		urea N 7 creat 16	93	134	41
Chronic nephritis, granular casts, albumin (FK)			98	146	48
Cancer prostate, anemia, 20 per cent Hb (Dr Russell "O'D")		NPN 82 creat 18	100	137	37
Infectious jaundice, albumin granular casts (Dr Stealy "D")		NPN 80 urea N 53 creat 12	100	137	37

*In parentheses, name of referring physician and identification of patient. All figures for blood chemistry in milligrams per 100 c.c. blood. F-W glucose by the Folin Wu method. L-B same by Benedict modification of Lewis-Benedict method. Alb albumin gr granular, h, hyaline c, casts.

TABLE V

CASES WITH NEGATIVE FINDINGS	DAYS	BLOOD CHEMISTRY	REDUCING SUBSTANCES		
			F W	L-B	Y REDUC TION
Toxic adenoma thyroid, albumin, granular casts, age 47 (28912)			100	99	-
Essential hypertension, trace albumin, casts, age 40 (28889)		urea N 22 25	128	135	7
Chronic nephritis, arteriosclerosis, myocarditis, albumin, few hyaline casts, age 67 Died (28411)			133	136	-
Arteriosclerosis, cardiac decompensation, albumin, hyaline casts, age 59 (28705)			156	156	-
Prostatic hypertrophy, age 89, trace albumin (28767)		urea N 15	129	121	- 8
Cholecystitis, trace albumin, casts, age 57 (31835)			166	150	-16
Pleurisy, fibroid tbc, albumin, casts, age 70 (28493)			132	119	-13

TABLE VI

CREATININE	Y REDUCTION	CREATININE	Y REDUCTION	CREATININE	Y REDUCTION
1	0	17	69	25	57
12	37	17	29	32	68
13	193	17	28	32	48
15	46	18	37	3.2	47
16	80	18	226	6	112
16	41	2	52	6	117
17	19	2	37		
17	62	25	18		

Table VII of 27 blood samples from 9 patients with nephritis shows the relation of the y-reduction to glucide X. Column 1 gives the blood glucose estimated by the Folin-Wu method, Column 2 the reduction by Folin-Wu after two hour fermentation by yeast expressed as glucose, "glucide X", the difference is the true (free) glucose. The average of glucide X in these nephritic bloods, 20.8 mg shows no increase over normal blood, nor is the true glucose high except in two samples (different cases). There follow the reduction by the Benedict modification, its increase over the Folin-Wu or the "y-reduction" the attempt to estimate the reducing bodies by the picrate method in blood from which the true glucose was first removed by yeast gave very irregular results (Column 7), perhaps an adsorptive effect on the picrate coagulum from yeast. Nevertheless the average was the same as the average of the sum of the two non-glucose reductions.

Somogyi¹ found glucide X uniform in quantity independently of the blood sugar level in health and disease, except in cases of severe diabetes when glucide X diminished as the high blood sugar persisted. Nor do we find glucide X

TABLE VII

F W GLUCOSE	F W AFTER YEAST FERMENTATION "GLUCIDE X"	TRUF GLUCOSE	L B GLUCOSE	Y REDUCTION	TOTAL, BOTH NON GLUCOSE RE- DUCTIONS	L-B AFTER YEAST
105	18	87	123	18	36	—
100	14	86	123	23	37	70
99	15	84	125	26	41	67
104	21	85	127	23	44	36
92	20	72	123	31	51	48
96	27	69	123	27	54	82
84	23	61	115	31	54	100
86	17	69	125	39	56	34
84	22	62	124	40	62	67
100	22	78	144	44	66	80
81	18	63	128	65	—	—
106	20	86	—	—	—	—
72	18	54	134	52	70	81
101	20	77	149	48	72	81
91	18	73	148	57	75	63
95	37	58	136	41	78	120
84	18	66	149	65	82	74
73	21	52	141	68	89	81
76	21	55	145	69	90	84
113	22	85	181	68	90	92
91	—	—	137	46	—	66
97	20	77	168	71	91	106
123	18	105	202	79	97	68
98	13	85	190	92	105	82
73	24	49	169	96	120	80
96	20	76	202	106	126	68
164	31	133	300	136	167	117
Average	20.8			55.5	76.7	77

higher in nephritis, although Linder, Hiller, and van Slyke,¹⁰ using the Folin-Wu method, found the nonglucose reducing body higher in cases of glomerulonephritis, about 40 mg, along with a lowered sugar tolerance and lower sugar threshold. Our findings in the blood of patients with nephritis are occasional high free glucose, normal glucide X, and a considerable increase over normal in such blood of a nonglucose reduction which we call the y-reduction, and which is present in much larger amount than glucide X (estimating as glucose) and may even be in greater reduction value than the glucose itself.

Fontes and Thivolle found glucide X consumed first under the influence of insulin, and more completely than glucose. Tests on a case of diabetes with chronic nephritis under treatment at the Naval Hospital, through the kindness of Dr. Hall, are recorded in Table VIII. The patient had a mild attack of coma during the time of observation. The remainder of fermentation, glucide X, did not disappear in this case when the blood sugar was high, but the y-reduction disappeared and at the highest glucose level, with large doses of insulin, the difference between the two blood sugar methods became a negative one, and this at a glucose level where the picrate method reads 3 per cent higher than the Folin-Wu and 24.5 per cent higher than the actual glucose content.

TABLE VIII

DIABETIC (MA) COUPTEST DR. HALL, U S N HOSPITAL				
DATE	F W	L B	Y REDUCTION	GLUCIDE X (F W AFTEP YEAST)
Oct 3	265	395	130	16
Oct 6	179	363	174	22
Oct 13	240	270	30	—
Oct 20	404	689	285	12
Oct 27	406	402	—	56
Nov 3	470	440	—30	20
Nov 17	420	555	135	22
Nov 24	393	516	125	
Dec 1	394	548	154	
Dec 8	437	660	223	
Dec 15	352	470	118	

The fact that the γ -reduction was demonstrated by a comparison of both reagent and oxidizing agents leads to inaccuracies which we tried to overcome by finding a more direct method. We found the Myers picrate method gave the same results in normal or nephritic bloods as the Folin-Wu, and used it frequently as a check, so that the γ -reduction may be measured by a comparison of picrate methods, the Benedict modification being sensitive to the γ -reduction substance together with glucide X and the true glucose, while the other two methods show reduction from only the latter two bodies. We later found the Ionesco¹¹ method sensitive to all the reductions like the Benedict modification, and by this method the γ -reduction can be demonstrated present in the tungstate filtrate. In a paper by one of us (R J P, read before the California State Medical Meeting, 1931), it was shown that this reduction is present as well in equal amounts in the trichloroacetic and m-phosphoric filtrates.

COMMENT

- 1 True glucose is often increased in nephritis
- 2 The nonfermentable remainder estimated by the Folin-Wu method, glucide X, is constant in health and disease. Contrary to most authors, we found glucide X occasionally fermented by yeast. The work of Hubbard and Deegan¹² suggests that it is a higher sugar.
- 3 The blood glucose may be estimated by either the Folin-Wu, the Myers, or Benedict copper methods without clinical error. When the glucose alone is to be estimated frequently we recommend the Folin-Wu technique as modified¹³ for use with 0.1 cc of blood. The Benedict modification of the Lewis-Benedict method should not be cited¹⁴ as a method for blood sugar as that will lead into greatest error. It is especially unfair to Dr Benedict who has substituted an accurate copper method¹⁵ for blood sugar.
- 4 We describe a reduction in blood which we call the γ -reduction, which varies in wide limits in health and disease, but averages much higher in the blood of nephritics and is highest in severe cases of nephritis. This reduction does not seem to have any relation to "creatinine." It may be due to several bodies and the reduction in nephritis may in part be due to a nitrogenous substance.

5 The question of glutathione occurs in connection with these results Benedict and Newton⁷ state that glutathione is one of the chief nonglucose reducing substances using their methods. They further state that it is present in blood to an extent of 50 to 100 mg per 100 cc and that with the Folin-Wu reagents it reacts in such a way as to give a reading in terms to glucose, corresponding to 0.2 the actual glutathione present, or in other words, an excess reduction of 10 to 20 mg per 100 cc. All these reactions are fundamentally of the oxidation-reduction type, and there is undoubtedly a fair degree of comparability between results gained by all methods.

Benedict¹⁶ states that in view of later work "a considerable portion of the saccharoid fraction of blood is not represented by glutathione." He likewise says "We are of the opinion that on the average glutathione accounts for little of the saccharoid content of blood." He shows that an average saccharoid content of 19.9 mg for twenty samples would require a glutathione content of 100 mg per 100 cc of blood, and adds that "There is not satisfactory evidence that blood contains upon an average one-half as much glutathione as this." In a footnote in this same article, he discusses a communication from Downes which gives an average glutathione content of 23.1 mg in six bloods by the Mason method. Benedict concludes the footnote with "Until more extensive figures are available for human blood by the Mason method and until the accuracy of the method has been further tested, we cannot draw definite conclusions concerning the glutathione content of blood."

Work is now in progress to determine the relation of glutathione to this y-reduction.

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A MANOMETER FOR MAGNIFICATION OF BLOOD PRESSURE TRACINGS*

By ROBERT K WEBSTER, A B, AND WILLIAM E FPL, INDIANAPOLIS, IND

INTRODUCTION

VARIOUS kinds of instruments for recording pharmacologic tracings make use of levers to magnify these changes when recorded on the kymograph. Whenever changes are relatively slight, as in kidney volume, spleen volume, or nasal mucous membrane volume, it is absolutely essential that these changes be

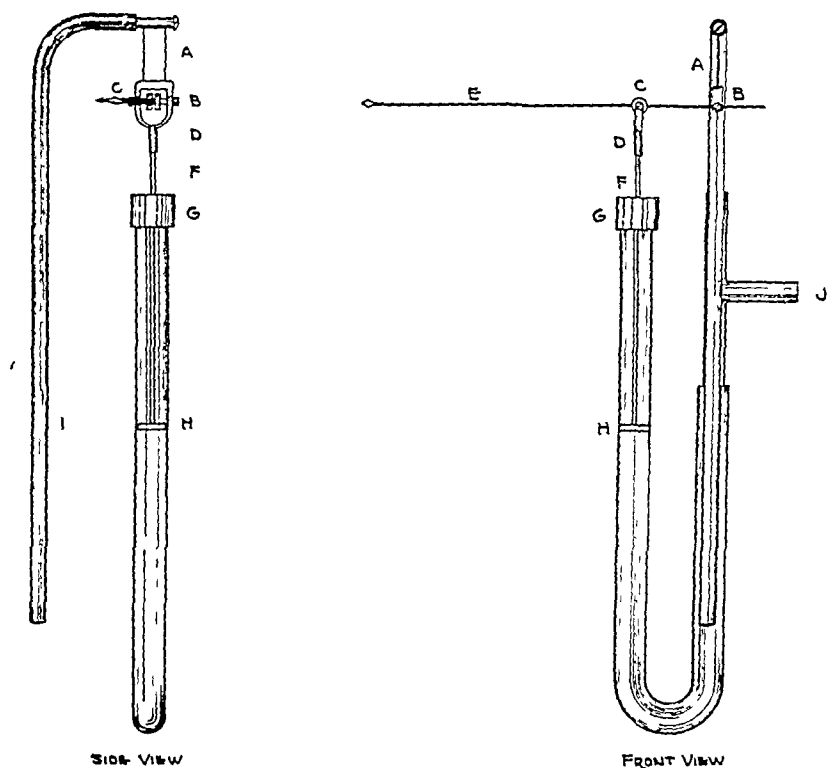


Fig 1

greatly magnified by the recording instrument. If this were not accomplished much greater difficulty in interpreting results would be experienced. It is believed that some such apparatus would be useful for comparative work on blood pressure, or for use when changes in blood pressure are very slight. As a result there has been developed a manometer which has been in use for several months and has been found very satisfactory.

*From Lilly Research Laboratories, Eli Lilly and Company.
Received for publication September 3, 1931.

DESCRIPTION OF MANOMETER

The usual mercury U-tube manometer was used and the levers were arranged as follows (Fig 1) *A* is a strip of copper $\frac{1}{2}$ inch by 1 inch, to the lower end of which was welded a needle-point bearing *B* such as is used on heart levers. This strip *A* was fastened at its upper end to a curved rod *I* for attachment to a ring-stand or other support. The lever *E* is of aluminum and passes through another needle point bearing *C*. This bearing is mounted in a Y-shaped holder *D* made of brass which was as lightly constructed as possible. A hole was drilled in the lower arm of the holder *D* in which was inserted another light aluminum wire *F*. The lower end of the wire *F* is fastened in an ordinary fiber float *H*. Connection to the artery is made in the usual manner from the arm *J*. The cap *G* of the manometer has an extra large opening for the passage of the wire *F* so that there will be no friction between wire and cap. There are three freely movable joints

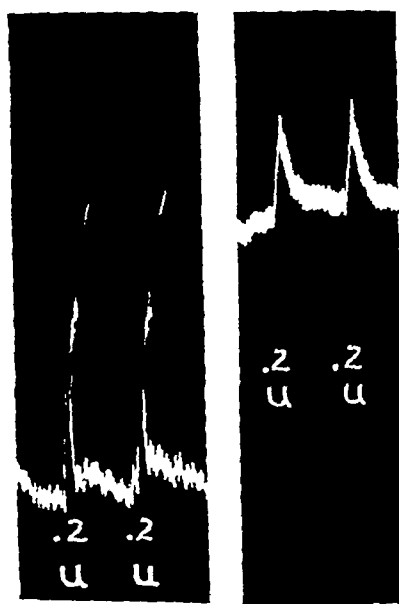


Fig 2.—Cat anesthetized with sodium amytal 65 mg per kg. Blood pressure recorded from carotid artery. Doses .02 unit of pituitary extract given intravenously.

in the apparatus at *A*, *B*, *C*, however, there is very little movement at *A*, as this plate moves only through a very small arc even with very great changes in blood pressure. These three movable joints permit the wire *F* to move freely and prevent friction.

DISCUSSION AND CONCLUSIONS

Success in pharmacologic assays depends largely upon the number of comparisons of a given drug on a single animal. The life of the animal may be prolonged by giving smaller doses and consequently more doses may be given. Since the recorded changes in blood pressure are relatively greater the accuracy of the assays increases accordingly. The exact change in blood pressure is easily calculated by determining the length of the lever arms and reducing

the result to millimeters of mercury. Furthermore, the apparatus has been used for both pressor and depressor drugs and has proved as satisfactory for one as for the other (Figs 2 and 3).

In conclusion, it is believed that this manometer has the following advantages over the old type manometer:

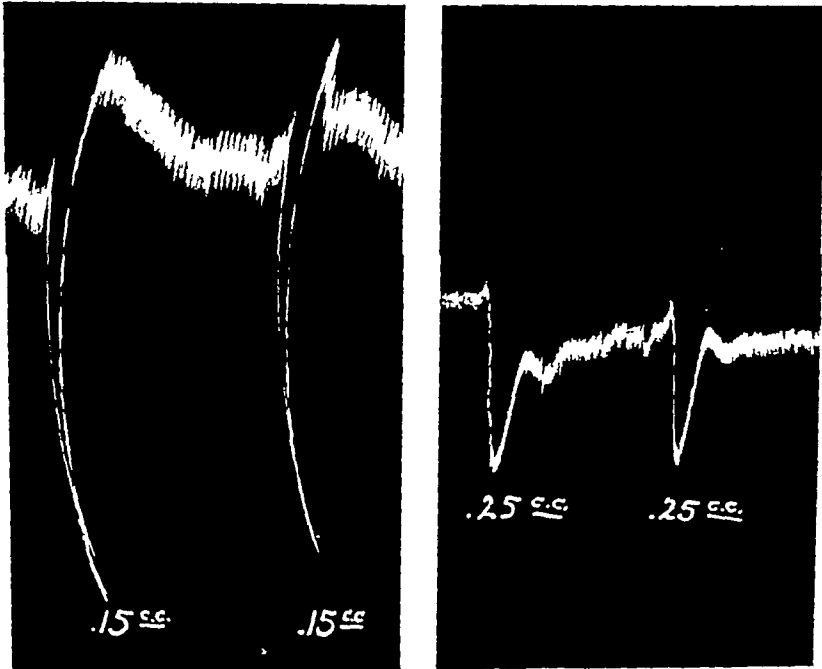


Fig 3—Cat anesthetized with sodium amytal 65 mg per kg. Blood pressure recorded from carotid artery. Doses 0.15 cc and 0.25 cc of 1-10,000 histamine dihydrochloride given intravenously.

- 1 Smaller doses of drugs may be given, thus prolonging the life of the animal.
- 2 Greater changes are produced on the kymographic tracing, thus permitting more accurate interpretation of results.
- 3 The magnification may be changed by changing the length of the lever arms, thus, very small changes of pressure may be greatly enlarged in the tracing.

A NEW TEST TUBE RACK FOR USE IN SEROLOGY AND BACTERIOLOGY*

By J. ARTHUR REYNIEPS, M. S., NOTRE DAME, IND

AT SOME time or another every technician has expressed dissatisfaction with the test tube racks now in use. The average test tube rack is bulky, nonadjustable and consequently will hold only a limited number of tubes of the same diameter or smaller than the diameter for which the rack is designed. Tubes held in larger holes than the size for which they are intended rattle disagreeably and have a tendency to slip from the rack. Furthermore the average rack tends to obscure if not to hide the contents of the test tube. Racks designed up to the present to overcome some of these difficulties are entirely too specialized and ex-

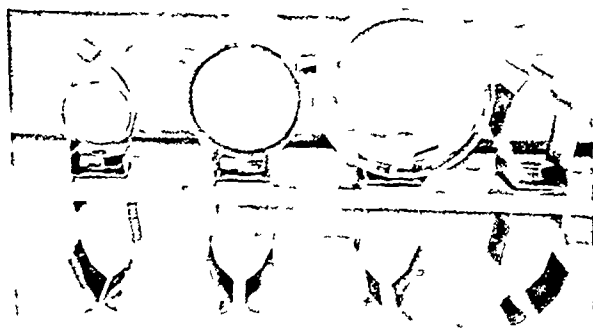


Fig. 1—Showing the arrangement of the clips on the clip bar and the method of holding test tubes of different diameters

pensive for the average laboratory. I believe that my rack has none of the disadvantages mentioned and that it has, moreover, decided advantages over any other rack I have seen.

The frame of the rack is constructed of a single piece of heavy brass or stainless steel, highly polished, bent into wings about two inches high. A bar of the same material as the frame is fitted into slots milled into the wings, and a drop of solder fixes the bar in place. Beneath this bar and parallel to it is a rod. Clips are mounted on either side of the bar. These clips are made of phosphor bronze and are designed for extreme flexibility and holding qualities. Experiment with over fifty different clip designs shows that great pressure is not required to hold the tubes firmly if the pressure is applied correctly. From Fig. 3 it can be seen that the test tube is held at six points of contact and not in a band-like grasp. Four of these points of contact press the tube back against the other points to hold it firmly in place. Flexibility is offered by the small indentations

*From the Bacteriology Laboratories, University of Notre Dame.
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at the base of the clip, the manner in which the clip is held to the bar and the length of the clip aims

The new rack has the following decided advantages over other racks. It is simple and compact in construction and inexpensive to make. It will hold twice the number of tubes in the space the older models required. It will hold firmly and securely any tube between $\frac{1}{4}$ inch to $\frac{3}{4}$ inch in diameter so that the contents

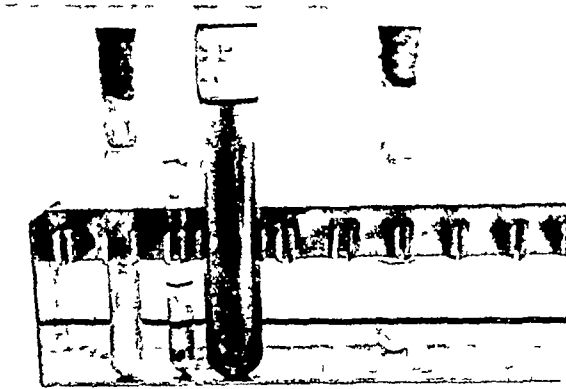


Fig. 2—Showing the perfect visibility of the tube contents obtained with this method of supporting test tubes

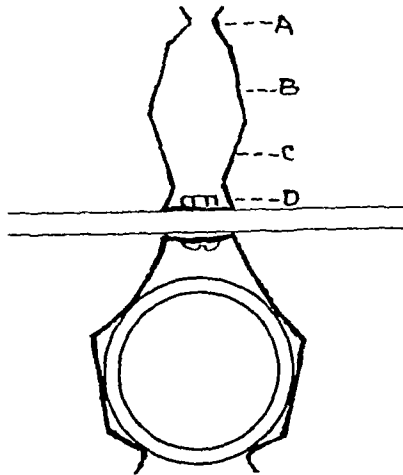


Fig. 3—Showing the principle on which the expanded and unexpanded clip holds the test tube

(A) Wing to facilitate the removal of the tube and to act as a point of contact. (B) Second point of contact with the test tube. (C) Point of contact against which the tube is forced by points (A) and (B). (D) Indentations in the base of the clip which with the base allow a maximum of expansion.

of the tube are visible along its entire length—a feature which will be appreciated by all those who have many tests to read. It will hold any shape test tube, will withstand autoclaving, and because of the simple construction of the base is firm and stable. Lastly the rack is “student proof,” holding up well under rough usage. The clips as experiment and constant usage in the bacteriologic laboratories of the University of Notre Dame for over a year has shown, do

not spread to any appreciable extent and if strained by a grossly oversize test tube can easily be replaced with a new clip or the old damaged clip may be pinched together by the fingers

The principle on which these racks are based, namely, the clips, can be used in many different ways. These clips may be adapted to racks for fermentation tubes, experimental museum exhibitions and culture libraries, to mention only a few examples

The racks are used in the same manner as the older models. Test tubes are pushed into the clips from above and may be drawn out the same way or quicker by simply pulling them out and away from the clip

A LABORATORY CHRONOGRAPH PROVIDING INTERMITTENT AND CONSTANT CURRENT FROM A DIRECT CURRENT LINE OF 110 VOLTS*

By O G HARNE, BALTIMORE, MD

A SIMPLE electrical chronometer operating upon 110 volts A C has recently appeared and was described by Chillingworth¹ In this chronometer a battery of cells is used to activate the laboratory apparatus In the present paper we offer a circuit operating upon D C lines, and used in our own installation

Wherever a 110 volt D C line is available, the circuit presented in Fig 1 will provide a convenient laboratory combination of intermittent and direct currents One or many lines to the laboratory tables may be had offering intermittent current through a "single" cycle circuit breaker serving as a chronometer giving impulses at second intervals A 1/60 cycle circuit breaker U^3 is propelled by the same motor providing minute intervals The potential through the breaker mechanism is controlled by P_2 , and is read off on V^1 Direct current is supplied to any number of tables through P_3 in quantities up to 3 amperes with a potential controlled by P_4 at each table, ranging from 0 to 15 volts (Equivalent to 10 dry cells) This unit of the combination is independent, and may be used alone as a complete substitute for batteries It may be used to replace the battery circuit in apparatus already installed, without changing the internal wiring,² or altering the commutator discs³

This circuit provides three lines to each laboratory table, (1) intermittent circuit, (2) ground, and (3) the direct current circuit Line 1 is made up of the two leads from the rotors of the circuit breakers fed through contact inserts C Line 2 is a common ground for both intermittent and direct current Line 3 arises from the arm of P_3 and feeds the table potentiometers The voltage is controlled by P_4 and read off on V_2 Potential changes are quickly made by rotating knob on arm of P_4

All the apparatus mentioned thus far may be purchased upon the open market at low cost, except the circuit breakers These are easily prepared Detail description is given in a previous article³ The number of lines through the circuit breakers is limited only by the number of contact inserts C The number of different time intervals possible with any one setting of P_1 is limited only by the number of circuit breakers shunted into the circuit of Line 1 The selection of time intervals depends upon individual needs but regardless of the intervals selected, the apparatus operates efficiently

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*From the Laboratory of The Department of Physiology University of Maryland
Received for publication September 4 1931

AN IMPROVED ETHER BOTTLE FOR ANIMAL ANESTHESIA*

BY R H K FOSTER, B CHEM ENG, CHICAGO, ILL

THE regulator herein described was developed for use with artificial respiration as provided by the C F Palmer (London, S W 2) artificial-respiration machine. This machine is provided with inlet and outlet valves arranged in such a way that air passes only in one direction through an ether bottle to a Y-tube attached to the trachea of the animal. The animal exhales through the other arm of the Y-tube which connects with the outlet valves in the machine. However the ether regulator can be used with other types of artificial-respiration machines. It can be used in spontaneous respiration if two one-way valves as used in metabolism apparatus are employed in the circuit to prevent re-breathing through the bottle.

Briefly, the advantages of this ether-air regulator are: a permanent record of the anesthesia may be made on the kymograph tracing, the ether vapor concentration is measured and easily adjusted at all times, a constant level of ether is maintained in the bottle thereby permitting uniform evaporation of the liquid, and finally, the supply is replenished without the disadvantage of removing the bottle lid frequently.

The figure illustrates the ether regulator with dimensions given in inches. The fittings are made of brass and soldered to a standard mason jar screw cap. A small tin can with a tightly fitting cork stopper is used as a reservoir for the ether. A hole is punched in the bottom and the can then soldered around this hole to the stopcock *L*. The other end of the tube from *L* runs 4 inches below the mason jar cap. This brings the open end of the tube from the reservoir to about the middle of a one-quart jar. As the liquid evaporates, air bubbles into the tube displacing ether from the reservoir. The stopper in the reservoir must of course be kept tightly inserted except when refilling when the valve *L* is closed.

Air from the artificial-respiration machine enters at *A* and goes to the animal from the exit *B*. Tube *A* is flattened at one end to form a slot one-eighth inch wide by one inch long. Tube *A* is soldered in place over a similar slot in the sleeve *E*. Sleeve *E* has an excursion of one inch on the vertical tube *H*. In the middle of *H* there is soldered a thin partition, *G*. Two slots, each one-eighth inch wide by one inch long are cut in tube *H* as shown at *F*. The sleeve *E* slides over these slots, being kept in position by the guide pin *O* as shown.

When the sleeve with the side arm *A* is in the midposition half the air will pass down into the ether bottle and half will pass upward around the by-pass *D*, the two streams again merging in *B*. By having the upper inch of tube *H* graduated in eighths, a scale is obtained whereby accurate adjustment of the mixture is easily effected. The sleeve is maintained in a fixed position by the heavy spring-bronze friction strip *K*.

*From the Pharmacological Laboratory, University of Chicago.
Received for publication October 8 1931.

The lug *J* is for the purpose of fastening a thread to a writing lever for recording on smoked paper. In operating it is desirable to place two styluses to mark base lines in order to indicate the maximum and minimum excursions of the ether-air-mixture recording lever.

The sleeve should fit snugly on *H*, yet slide with ease. The by-pass *D* simply slips over the tops of tubes *H* and *N*. *M* is a flat brass strip to which the various tubes are soldered and which in turn is soldered to the mason jar cap. It also facilitates screwing the cap on the jar. Tube *A* must be three-fourths of an inch in diameter, so that the one end when flattened will have a slot one inch long.

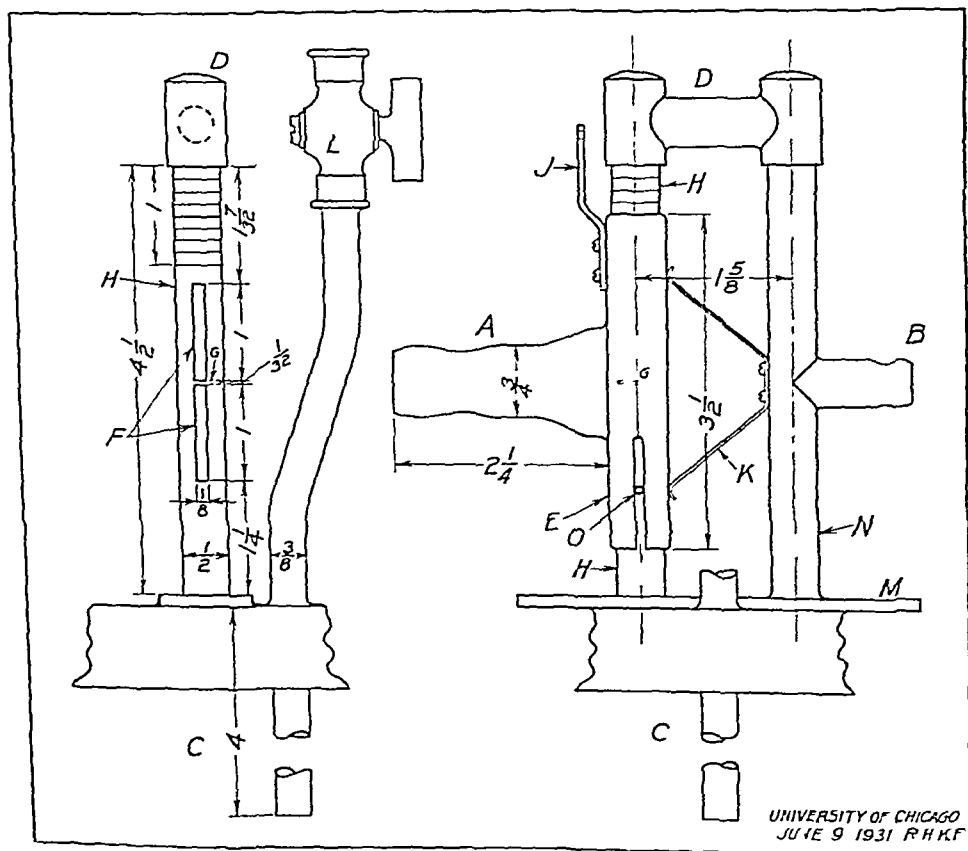


Fig 1

The sleeve is of such length that when completely lowered all the air passes through the ether bottle and when completely raised none passes through

In order that the adjustment of the writing lever will not be disturbed the ether bottle is anchored to a ringstand by clamps

SUMMARY

1 An ether-vapor regulator is described by means of which a permanent record of the anesthesia is obtained. Changes in circulation or respiration can be correlated with any changes in the ether-vapor concentration.

2 The proportion of ether-vapor to air can easily and fairly accurately be regulated

3 A constant ether level can be maintained in the bottle, thus eliminating variations of ether-vapor concentration due to changes in liquid level

4 The ether bottle can be refilled without removing the mason jar cap. Thus in a prolonged experiment or series of experiments the jar can be anchored in place, obviating the necessity of readjusting the recording level each time the bottle is refilled

5 The device can be used in studying effects of changes in vapor concentration and is suited to other volatile anesthetics such as chloroform

6 The device is designed for one-way air passage, thus eliminating rebreathing expired air from the dead space and in addition using less ether

In conclusion, the author wishes to express thanks to Dr H B Van Dyke and Dr A J Carlson for their cooperation in making suggestions and to Mr Gus Lutz whose mechanical skill and ingenuity aided materially in producing a satisfactory working mechanism

METHOD FOR STAINING FECAL PROTOZOA*

BY RAWSON J. PICKARD, M.D., AND CLARA RICE, R.N., SAN DIEGO, CALIF.

THE case of a physician who for years had had pathologic fatigue, brief diarrheas, and abdominal distress, and who had examined his own stools many times both in fresh preparations and with iodine, with no other finding than innumerable yeast cells, recently reemphasized on us the necessity for making all examinations for fecal protozoa from wet fixed preparations stained with iron hematoxylin. The innumerable "yeast cells" proved to be equally numerous cysts of the small race of *E. histolytica* (*E. Hartmanni*).

We have long used a technic modified from that given by Langeron,¹ by a rapid method, obtaining preparations suited for species diagnosis, and by a slower technic, getting regularly pictures fully as detailed as those obtained by the somewhat lengthy Schaudinn fixation. The examination for proper differentiation in creosote gives a clarity which is not possible with examination in water and the species diagnosis may be made then if permanent slides are not desired. If further differentiation is required the creosote is readily removed with alcohol or if differentiation has gone too far the steps may be retraced further back. Also, due to the difficulty of obtaining the necessary quantities of ethyl alcohol, we found that the ordinary "completely denatured alcohol, Formula 1," is a perfectly satisfactory substitute after it is clarified and purified by distillation over NaOH, giving a water clear product of 95 per cent alcohols.

TECHNIC

- 1 Fixation in alcoholic Bouin's solution, one hour, room temperature
- 2 Harden five minutes 95 per cent alcohol
- 3 Seventy per cent alcohol, five minutes, may leave in this indefinitely
- 4 Wash in two changes water (tap)
- 5 Ferric alum 3 per cent 15 minutes at 32° C, rapid technic (12 hours room temperature for slow technic for precision of detail)
- 6 Wash, two changes water (distilled)
- 7 One per cent hematoxylin 15 minutes at 32° C, (12 hours room temperature, slow technic)
- 8 Wash in water
- 9 Differentiate in 3 per cent ferric alum at 32° C, (at room temperature, slow technic)
- 10 Wash in water
- 11 Wash in 95 per cent alcohol
- 12 Clear in creosote drain off excess examine with 50X oil immersion using a cover glass to protect the lens from creosote. If permanent preparation is

*Received for publication September 17, 1921

desired and the differentiation is correct for the protozoa found present, remove the cover

13 Clear in xylol, mount in xylol balsam

Age the 1 per cent hematoxylin six weeks in sunlight, add a crystal of thymol to preserve. Alcoholic Bouin's solution made fresh daily from 30 c c of 80 per cent alcohol saturated with picric acid, 12 c c formalin (40 per cent), and 3 c c glacial acetic acid. Coplin jars for various solutions. For the fixation the flat Laveran "boxes" are preferable. Differentiation varies from a few seconds for flagellates to several minutes for the larger amebic cysts, it must be controlled under the microscope.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

B. DIPHTHERIAE Stain for, Gutstein, M. and Neisser, H. *Centralbl. Bakteriol.* 108:253, 1928

- 1 Stain two to three minutes with 1 per cent aqueous Azure II.
 - 2 Wash in water
 - 3 Differentiate with 3 per cent acetic acid (one to three seconds)
- Granules are reddish violet, bodies blue
- or
- 1 Stain two to three minutes with 1 per cent aqueous Azure II
 - 2 Wash with water
 - 3 Wash with aqueous chrysamine
- Granules are black, body yellow

ALLERGIC ASTHMA Nonspecific Desensitization Therapy in The Eosinophilic Index as a Guide to Intramuscular Injection of Venom Protein, Spangler, R. H. *Arch. Int. Med.* 36:779, 1925

It would be of value if a method could be established for regulating the strength of the dose and the frequency with which a protein injection should be given in order to keep a patient nonspecifically desensitized.

Spangler, who has used intramuscular injections of venom protein (crotalin) for many years, suggests a study of the eosinophiles in the differential count (eosinophilic index) as a satisfactory guide.

As a rule, the highest rise in eosinophiles after crotalin injections ($\frac{1}{400}$ to $\frac{1}{50}$ grain) occurs by the second or third day.

It is his practice not to repeat an injection if the eosinophile count has not dropped to normal or to the preinjection level by the fifth day after injection. Moreover, it is wise not to increase the dose if any given dose is producing an eosinophilia of 8 to 10 per cent by the second or third day after injection.

KOTTMAN REACTION Diagnostic Value of the Kottman Reaction in Thyroid Dysfunction. *Am. Jour. Med. Sc.* 172:84, 1926

From a study of 101 cases, including hyperthyroidism and various pathologic conditions, Katavama concludes that

It is generally conceded among physiologists and clinicians that the determination of the basal metabolic rate is the most dependable laboratory index of thyroid activity. Increased thyroid secretion produces a lowering of the tolerance for carbohydrate, but there are numerous other conditions in which the tolerance for carbohydrate may be diminished. Hence the occurrence of high blood and urine sugar curve after the ingestion of glucose is not in itself indicative of hyperthyroidism. In hyperthyroidism, however, the blood and urine sugar curves after glucose furnish information concerning a phase of carbohydrate metabolism which is not gauged by the basal metabolic rate.

The basis of the Kottmann reaction is obscure, and hence it is difficult to say in what manner thyroid activity influences it.

BRAIN TUMOR Yellow Spinal Fluid Associated with Tumor of the Brain, Comfort, M. W Arch Neurol and Psychiat 15 751, 1926

The present review has led to the belief that a definite localizing and prognostic significance is attached to the presence of xanthochromic fluids in cases of brain tumor, and that the following conclusions, although based on the study of a comparatively small group of cases, are justified

Yellow spinal fluid associated with tumor of the brain occurs much more frequently than the reported cases indicate. In the present series, xanthochromia occurred in approximately 20 per cent of the cases of tumor of the brain.

Xanthochromic spinal fluid associated with a tumor of the brain indicates the involvement of the ventricle or external surface of the brain, and that the tumor is sufficiently vascular and soft, or so surrounded by engorged vessels, as to permit hemorrhage or transudation into the cerebrospinal fluid.

The available evidence points to a hemolytic origin for the xanthochromia accompanying tumor of the brain, a serous origin, while theoretically possible, has not been proved.

The hemorrhages accounting for the coloration are conceived of as being scanty and repeated.

The number of erythrocytes present is a measure of the softness and the vascularity of the tumor.

Xanthochromia combined with the presence of many erythrocytes, indicating the presence of a soft vascular tumor prone to hemorrhage, should greatly increase the risk of, and possibly contraindicate, those operative procedures which markedly reduce the intraventricular pressure.

DIABETES Relation of Abdominal and Rectal Infections to the Pathogenesis of Diabetes Mellitus, Visher, J. W. Am Jour Med Sc 171 836, 1926

The underlying pathologic change in diabetes in many cases is a pancreatitis.

This pancreatitis may originate in acute infectious diseases, and from hematogenous focal infections.

Five cases of diabetes mellitus apparently secondary to abdominal and rectal infections are reported, with improvement following surgical intervention.

The opinion is ventured that in these cases the infection reached the pancreas through the lymphatics, either directly or by way of the portal circulation.

The conclusion is suggested that abdominal and rectal infections are important etiologic factors in the etiology of diabetes mellitus.

MERCUROCHROME 220 SOLUBLE The Use of, in the Treatment of Infectious Diseases of the Skin, Young, H. H., Hill, J. H., and Denny, W. L. Arch Dermat and Syph 13 465, 1926

The following cases in which mercurochrome was used intravenously are reported.

Twenty-four patients with erysipelas, of whom twenty (83.3 per cent) were cured or greatly improved, eleven patients with furuncles and carbuncles, of whom ten were cured and one greatly improved, four cases of chancroidal ulcerations, all of which healed rapidly, thirty-six patients with cellulitis and abscesses, of whom twenty-one (58.3 per cent) recovered promptly with no other treatment, and nine of whom (25 per cent) showed marked improvement and recovery after mercurochrome therapy, other treatment being also given, making a total of thirty cases, or 83.3 per cent, cured or greatly improved, two patients with gas gangrene, in both of whom the infection was eliminated, one patient with diabetic gangrene, in whom an accompanying infection was checked, three patients with pemphigus, all of whom showed marked improvement, two of them so far without relapse, one with relapse, four patients with psoriasis, two of whom are apparently cured, one markedly improved, and one improved, but with a relapse, one patient with eczema, temporarily improved, one patient with syphilis with exterior skin lesions, in whom the lesions were healed,

forty four patients with leprosy, twenty eight (63.5 per cent) of whom were improved and with remarkable disappearance of the skin ulcers

HYPERGLYCEMIA The Relative Blood Volumes in Diabetes Mellitus, Foshay, L. Arch Int Med 36 889, 1925

Poluria and exsiccation occur in diabetes in proportion to the severity of the disease. It seems probable, then, that diuresis and renal permeability have some influence in determining the course. If so, diabetes in the young should show more evidence of blood and tissue dehydration than in the elderly in whom the disease is less apt to be as severe.

The studies of Foshay were designed to secure evidence as to the correctness of these suppositions.

Tissue exsiccation was determined by clinical observations of the skin, subcutaneous tissues, and mucous membranes, anhydremia and hydremia by the relative volumes of serum calculated by the method of Stewart.

Studies were made of 13 young diabetics, 15 arteriosclerotic diabetics presenting acute exacerbations, and 8 patients who had been long under treatment, as a result of which the following conclusions are presented.

Diabetes mellitus produces marked changes in water distribution and in the total water balance of the body. The degree of these changes is modified by the degree of hyperglycemia and the rate of onset, and their character by vascular disease and renal permeability.

Reduction of hyperglycemia or the action of insulin may produce either a decrease or increase in the concentration and viscosity of the blood depending, in either case, on the antecedent water content.

GNORRHEA The Diagnosis of, Through Intracutaneous Vaccine Injections, Kohler, H. Ztschr f urol Chir 19 54, 1926

As a result of his experimental studies Kohler concludes that the induration of the skin produced by the intracutaneous injection of 0.5 c.c. of gonococcus vaccine containing 3,000 to 10,000 organisms per cubic centimeter, if it persists more than twenty four hours, is specific for gonorrheal infection, either present or recent.

BLOOD Physical and Chemical Studies of Human, from Cases of Diabetes Mellitus, Foshay, L. Arch Int Med 37 18, 1926

The following studies were made on defibrinated blood.

- 1 Erythrocyte count in duplicate and checking to 250,000 per cu mm
- 2 Electrical conductivity of whole blood and serum, the result expressed as specific conductivities ($K \times 10^4$ at $50^\circ C$)
- 3 Relative volumes of serum and erythrocytes by calculation
- 4 Average erythrocyte volume the relative volume of erythrocytes in cu. mm divided by the number of erythrocytes per cu mm. Expressed as cubic microns
- 5 Glucose in serum and whole blood determined by Folin Wu technique
- 6 Chlorine determined by Whitehorn's method (Jour Biol Chem, February, 1921, xlv, 449)
- 7 Corpuscular chlorine and corpuscular glucose concentrations

$$\% \text{ in cells} = \frac{\% \text{ in whole blood} - (\text{serum volume } \% \times \% \text{ in serum})}{\text{cell volume } \%}$$

- 8 Grams glucose and grams chlorine per erythrocyte

$$\frac{\text{Grams substance per 1 cu mm cells} \times \text{cell volume } \%}{\text{Number of cells per cu mm}} = \text{Grams substance per cell}$$

Summary The conductivity of whole blood and serum varies inversely as the concentration.

In diabetes a "conductivity chloride discrepancy" occurs, the cause of which is not known.

The normal erythrocyte volume is approximately 76 cubic microns.

In all the young diabetic patients and in two of the arteriosclerotic patients with acute exacerbations, the volume average 85 to 90 cubic microns. With recovery the volume returned to normal.

The order of events would seem to be as follows. In young patients hyperglycemia produces a dehydration of the blood and of the fixed tissues, thus causing a nonvolatile acidosis with consequent increase in erythrocyte volume by reason of water transference. This may be considered the predisposing cause of diabetic acidosis. As cellular metabolism becomes more and more abnormal there is a production of ketone acids in increasingly larger amounts, thus further diminishes the plasma alkaline reserve. In cases of severe acidosis with rapid onset, no doubt both processes become important factors.

In elderly, arteriosclerotic patients, hyperglycemia does not result in dehydration and acidosis, hence no water transference from the plasma to the erythrocytes and no increase in erythrocyte volume occur. The patients live in relative comfort and are not in constant danger of acidosis and coma. If a serious dietary indiscretion or acute infection supervenes, then dehydration, acidosis, and coma occur just as in the young diabetic patient.

In general, the corpuscular glucose is usually a little less than the concentration in the serum, however, with increasing hyperglycemia the greater portion of the glucose is found in the serum and conversely.

Concerning the distribution of chlorine, the only constant finding in diabetes is that in the presence of hyperglycemia the chlorine concentration of whole blood is always diminished. A consistent relationship between hyperglycemia and corpuscular chlorine was not found.

In untreated, arteriosclerotic diabetic patients there was no evidence of diminished blood alkalinity unless the patient was in coma.

WASSERMANN REACTION The Rational Use, Haythorn, S. R. Ann Clin Med 4 493, 1925

Haythorn uses the laboratory requisition below in order to acquire reliable statistics.

WASSERMANN REQUISITION

Please fill out for research data. The laboratory reserves the right to withhold the report until the requisition blank has been filled out.

Blood

Name _____ Spinal Fluid _____ Date _____ Hour _____

Hospital Address _____ Physician _____

Nationality _____ Sex _____ Married _____ Age _____

Chief Complaint _____

Clinical Diagnosis _____

Is the patient suffering from any febrile disease? _____

Clinical indications of specific disease (place circle around type indicated)

None, Questionable, Primary, Secondary, Tertiary, Hereditary, Cerebrospinal

Duration of suspected specific disease _____

Results of previous Wassermann tests _____

Approximate number of arsphenamine treatments _____

How long since last treatment? _____

Other antiluetic treatments _____

If your reason for requesting the test has not appeared in filling out the above blank, will you kindly state it here _____

Pregnant _____ Month _____ Miscarriages _____

Postpartum _____ Days _____

TUBERCULOSIS AND ASPERGILLOSIS Aspergillosis of the Lungs and Its Association with Tuberculosis, Lapham, M. E. Jour Am Med Assn 87 1032 1926

Lapham summarizes from the literature the reports of pulmonary aspergillosis in human beings, calls attention to the difficulty of differentiating this condition from tuberculosis and

expresses the belief that it is a more frequent complication or concomitant of tuberculosis than is recognized. She argues for routine cultures of the sputum in tuberculosis and says

"Here is a disease that is capable of causing pleurisy, acute and chronic, bronchitis acute and chronic, pneumonias, acute and chronic, emphysema, bronchiectasis, atelectasis, sclerotic fibrosis, tubercles, cavities, endarteritis, thrombosis, infarcts, hemorrhages, asthma. Would it be strange if such a disease should seriously complicate or even inhibit recovery in a case of tuberculosis?"

She concludes that we are thoroughly ignorant of the frequency of aspergillosis both as a primary and as a secondary disease.

We have no idea how much aspergillosis of the lungs predisposes to tuberculosis in human beings or in cows.

We do not know how much it impedes or even inhibits recovery in cases of tuberculosis.

We have no idea whatever as to its association with acute respiratory diseases.

We know that it affects cows much as it does human beings.

We know that it gives the same reaction to tuberculin that tuberculosis does.

Should we apply this knowledge to the study of the tuberculosis of dairy herds?

In order to gain adequate information as to the frequency and importance of this disease, should not a systematic research study be made?

1 By determining the percentage of aspergillosis cases among the tuberculosis cases in the large tuberculosis sanatoriums.

2 By determining the percentage of cases of aspergillosis in the lungs at necropsies.

3 By determining the percentage of cases of aspergillosis in cases of respiratory diseases.

4 By applying these principles to dairy herds.

FOREIGN BODIES IN THE LUNG Pathologic Changes in Lung Tissue as the Result of Foreign Bodies of Long Sojourn, Manges, W F Jour Am Med Ass'n 87 987 1926

For the purpose of this paper, a foreign body is one of long sojourn after it has been present in the air passages for two months or more and up to as many as thirty-five years, except that in one or two instances the sojourn has been less than two months but the pathologic changes are unusual. Serious pathologic changes do occur at times in cases of much shorter duration, but these are more or less constantly the acute type, such as infection, emphysema or atelectasis, with which physicians are quite familiar.

An aspirated foreign body in any portion of the tracheobronchial tree will sooner or later cause extensive permanent injury.

There is great variation as to the length of time a foreign body may be present before causing extensive changes, but those that interfere with drainage do, as a rule, cause injury early.

The permanent pathologic change is distal to the point at which drainage is blocked. The foreign body is at or distal to this point.

The end results are in the nature of atelectasis, fibrosis, bronchiectasis and chronic abscess, with varying quantities of purulent exudate. Hemorrhage is common, tuberculosis is often suspected but is rarely present. The other lung remains remarkably free from pathologic change.

Manges believes that many of the one-sided, chronic, basal infections are the result of foreign body, regardless of history or of roentgenographic shadow of foreign body. Such lesions should at least be investigated bronchoscopically, and many should be treated in this manner.

FOREIGN BODIES IN AIR PASSAGES Live Fishes Impacted in Food and Air Passages of Man, Gudger E W Arch Path and Lab Med 2 355 1926

Gudger, who is bibliographer to the American Museum of Natural History, has collected from the literature all the reports of impaction of live fishes in the human throat and air passages.

The paper is most readable and of extreme interest but does not lend itself to abstraction

CARCINOMA Grading the Malignancy of Carcinoma, Grading and Practical Application, Broders, A. C. Arch Path and Lab Med 2 376, 1926

Broders uses the following classification

If an epithelioma shows a marked tendency to differentiate, that is, if about three fourths of its structure is differentiated epithelium and one fourth undifferentiated, it is graded 1, if the differentiated and undifferentiated epithelium are about equal, it is graded 2, if the undifferentiated epithelium forms about three fourths and the differentiated about one fourth of the growth, it is graded 3, if there is no tendency of the cells to differentiate, it is graded 4. Of course, the number of mitotic figures and the number of cells with single large deeply staining nucleoli (one eyed cells) play an important part in the grading.

He further modified this conception as follows

Instead of a Grade 1 epithelioma in which about three fourths of the cells are differentiated and one-fourth undifferentiated, should be substituted a Grade 1 epithelioma, one in which differentiation or self control ranges from almost 100 to 75 per cent, and undifferentiation from almost nothing to 25 per cent, a Grade 2 epithelioma, one in which differentiation or self control ranges from 75 to 50 per cent, and undifferentiation from 25 to 50 per cent, a Grade 3 epithelioma one in which differentiation or self control ranges from 50 to 25 per cent, and undifferentiation from 50 to 75 per cent, and a Grade 4 epithelioma, one in which differentiation or self control ranges from 25 per cent to practically nothing, and undifferentiation from 75 to practically 100 per cent. So far as an estimation of the ultimate result is concerned, this revision will have no effect on Grades 1 and 2, but will affect slightly Grades 3 and 4, because a small percentage of neoplasms, formerly classified Grade 3, will now be classified Grade 4. In other words, the most malignant of the Grade 3 neoplasms will be classified in Grade 4.

He calls attention to the practical application of this procedure in these words

"Turning to the practical side of the grading of cancer, it is well known that cancer of Grade 1 shows practically no tendency to metastasize, and therefore in dealing with such neoplasms it does not seem necessary to remove the regional lymph nodes. This saves the patient unnecessary operative procedures. As practically all cancers of Grade 4 with metastasis prove fatal sooner or later, the patients should not be subjected to an operation involving the regional lymph nodes unless they are in close proximity to the primary growth, cancer for the stomach for example. Judd New, and Figs believe it is useless to perform block dissection of the neck in the presence of a Grade 4 epithelioma of the lip, tongue, cheeks, floor of the mouth or antrum, etc. In cases of cancer of Grade 2 and in a certain proportion of those of Grade 3, with metastasis, removal of the regional lymph nodes offers a permanent cure in a fair number of cases, as evidenced by the fact that ten (33.3 per cent) of thirty patients with squamous cell epithelioma of the lip of Grades 2 and 3, with metastasis in one group of submaxillary lymph nodes, were living and well on an average of six and one fifth years after removal of the nodes.

OZENA, BACTERIOLOGY Bacillus Ozena Foetidæ, Perez, and Bacillus Proteus in Ozena, Michailoff, A. Bull. Johns Hopkins Hosp. 39 158, 1926

Ozena is a chronic disease of the nose characterized by a mucopurulent discharge, crusts, a specific fetid odor, and atrophy of the turbinate bones.

A bacteriologic study of twenty eight cases. The bacillus described by Perez was isolated in only seven cases. A bacillus of the proteus group was also found with sufficient frequency to warrant study of its connection with the disease.

Bacteriologic and immunologic studies are reported in detail and the author's conclusions are as follows:

Although the cultural and fermentative properties are insufficient to identify Perez bacillus as atypical Proteus, we are justified in thinking that it constitutes a sub group which

has lost some of its fermentative properties, a frequent occurrence with the most typical proteus, and that *B. ozaenae liquefaciens* Shiga is a typical *B. proteus vulgaris*.

The agglutination and agglutinin absorption tests show Perez bacillus to be very closely related to *B. proteus*, Perez X is intermediate between the two species, having agglutinative properties as strong for Perez bacillus as for *B. proteus*. Each bacillus of this group has a serologic individuality more or less close to the Perez or proteus species. Of the fourteen bacilli, no two are identical. Agglutinin absorption shows clearly that the motile strains, *B. ozaenae liquefaciens* Shiga, and *B. proteus vulgaris*, as a rule, have their coagglutinative branches reduced or removed when absorption is done with the motile bacillus, and vice versa, they are more closely related to each other than to the nonmotile Perez species and can be identified as belonging to the same group as *B. proteus*.

Complement fixation shows that the two varieties, motile and nonmotile, have a very close relation. Some Perez and Proteus bacilli have identical properties as antigens against a given serum and this reaction appears to show closer relationship than the agglutination or agglutinin absorption test. There is no correlation between the complement fixation and agglutination test.

The more specific flocculation reaction also shows the organisms to be very closely related, but only Perez II and Hofer's Perez bacilli have identical properties, while of these only the latter shows flocculation with *B. proteus*.

B. proteus, *B. perez*, and *B. ozaenae liquefaciens* have identical pathologic properties. Each produces the four different types of infection: (a) toxico-septicemic congestive and hemorrhagic lesions, (b) chronic pyemic, mucosal, and endothelial exudative and proliferative lesions, (c) local exudative lesions, and (d) special necrotic lesions due to complex pathologic processes.

All the strains studied produce a toxin identical with that of Hofer's Perez bacillus and *Proteus vulgaris*.

Cross immunization shows that Perez bacillus and *B. proteus* have identical immunizing properties, protecting completely or producing sufficient protection to indicate their identity or very close relationship.

On the basis of the above conclusions it follows that the Perez bacillus is a member of the large group of Proteus bacilli.

The etiologic relation of the Proteus Perez group to *ozaenae fetida* has been suggested by many workers because of the frequent finding of these organisms in the nasal discharge of such cases. The Proteus group exhibits an affinity for the blood vessels and mucosae, has pathogenic power, producing chronic necrotic lesions and nasal ulceration and discharge. We never produced green crusts or atrophy of the turbinate bones in the rabbit, and therefore we cannot conclude that in human beings *B. proteus* produces *ozaena fetida*, and that this bacillus acts as a primary etiologic agent. The frequency of its occurrence and the specific malodor found in the cultures show that *B. proteus* is involved in the pathogenesis of *ozaena*, and whether implanted primarily or secondarily, is the cause of the fetor, discharge, and ulceration.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor,
Dr Warren T Vaughan, Professional Building, Richmond, Va

Histopathology of Skin Diseases

IT IS, of course, fortunate for the patient that few die as a result of diseases of the skin per se, but unfortunate from a scientific standpoint in that this fact has undoubtedly retarded advancement in the understanding of these conditions.

There are volumes galore upon the symptomatology of diseases of the skin and innumerable compendiums of formulae for their treatment. But there are few volumes, indeed, which present in an authoritative and comprehensive way the details of the histopathology of diseases of the skin.

Dr McCarthy and the publishers are to be congratulated, and the thanks of the medical profession are due to Mr Truxton Beale through whose generosity the publication was made possible, for the eminently successful completion of this publication.

Without doubt, this book will become an authoritative reference for the profession at large and will establish a standard for similar works of the future.

Dr McCarthy, in his presentation of the subject shows not only a thorough comprehension of its many ramifications, evidently based upon an extensive and practical experience, but also a thorough knowledge of the literature.

As is well known, a well conceived and well executed illustration is often more informative than pages of descriptive text. This book, therefore, is well and profusely illustrated many of the plates being in color. While microphotographs are not neglected, great reliance has wisely been placed upon careful, accurate, and excellent drawings in which the essential features can be clearly shown and the unessential minimized.

The reproduction of the drawings and especially of the colored plates are exceptionally well done and when, as in this book, both text and illustrations are equally well done and truly complementary, the result is a volume of the greatest value to students of the subject.

This book may be recommended without reserve as a most valuable and useful contribution to an important and, in many ways, obscure subject.

The Regulation of Size As Illustrated In Unicellular Organisms†

SIZE is one of the chief properties of living organisms and the problem of size regulation is one to which the attention of science has long been turned and in connection with which a number of more or less directly related observations have been recorded.

In this book the pertinent data in this field have been collected, arranged, and weighed by Professor Adolph so that the investigations of many observers are here outlined as a coordinated whole.

The book covers a field as yet but little tilled and constitutes a contribution of distinct interest to biology and kindred sciences.

*Histopathology of Skin Diseases By Lee McCarthy M.D. Associate Clinical Professor of Dermatology Georgetown University Medical School, etc. Cloth 513 pages 251 illustrations many in color. The C. V. Mosby Co. St. Louis Mo.

†The Regulation of Size as Illustrated in Unicellular Organisms By Edward A. Adolph, Associate Professor of Physiology in the University of Rochester. Cloth 230 pages 66 figures. C. C. Thomas Springfield, Ill.

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EDITORIAL

Protein Digestion and Food Allergy

SINCE men first reached that stage where they could compare experiences, food idiosyncrasy has probably been a recognized entity. The interpretation of these isolated individualistic manifestations has necessarily varied at different epochs, dependent upon the medical teachings of the day, some of which indeed have persisted and still color our dietary prescriptions. Such for example is probably the popular belief in poisonous combinations of otherwise harmless foods, as sea foods and cream eaten at the same meal. Many of the arbitrary diets for gall bladder disease and colitis still to be found in modern textbooks would appear to be based upon cumulative past experience with cases of food idiosyncrasy.

The striking picture of respiratory anaphylactic death in the guinea pig led to the suggestion that bronchial asthma might be associated with protein sensitization. Knowledge that experimental anaphylaxis may be produced with food proteins resulted in the demonstration that alimentary allergens as well as inhalant allergens could produce allergic manifestations. But at the beginning the

only manifestations which were universally accepted as indubitably allergic were bronchial asthma, vasomotor rhinitis and urticaria. After it had been demonstrated that the allergic response consists primarily in smooth muscle spasm and serous exudation, and that although the reaction is predominantly respiratory in guinea pigs, it is predominantly circulatory and hepatic in rabbits, and gastrointestinal in dogs, horses, hogs and cattle, the conception gradually gained acceptance that a great variety of organs and tissues might be involved in the anaphylactic response and may therefore become responsible for symptoms.

Even in the guinea pig, although the startling picture manifests itself in the respiratory system, other tissues take part in the response. The experiments with sensitized guinea pig uterus are well known. Kendall and Shumate¹ have recently demonstrated and studied quantitatively the anaphylactic reaction in the intestinal musculature of guinea pigs. They found greatest anaphylactic response in the lowermost portions of the small intestine, gradually diminishing upwards, with least response in the lower portion of the duodenum. In the upper duodenum anaphylactic sensitivity was again increased. The participation of the intestinal tract in the allergic response appears to have been experimentally demonstrated. Indeed Templeton and Bollens² have devised a method for the study of anaphylaxis, comparable to the uterine strip method, in which they measure increased tonus within the rectum and lower colon as evidence of anaphylactic response.

Studies of gastrointestinal absorption, in the production of experimental anaphylaxis, have necessitated a thorough revision of our understanding of the physiology of digestion. No longer can we state that proteins are completely broken down into their constituent amino acids in the intestines, and absorbed through the mucosa as such.

Assuming that sensitization may occur through intestinal absorption, how does it happen? Schloss and Worthen³ found by precipitin and anaphylactic tests applied to the urine, that the intestinal tract of normal infants is impermeable to undigested foreign protein. In the presence of gastrointestinal disturbances however, protein was found to be absorbed, either undigested or partially digested and excreted in the urine. This seemed a simple explanation. We become sensitized to food protein as a result of gastrointestinal disturbances which allow the passage of undigested allergen into the blood.

Hettwer and Kriz⁴ substantiated these findings by sensitizing guinea pigs to horse serum following its introduction into a temporarily ligated loop of small intestine. They found that stasis and increased intramintestinal pressure were necessary to promote absorption. They obtained similar results following chemical irritation without stasis, by the introduction of horse serum into the unligated intestine together with small amounts of sodium fluoride. Not only did they sensitize guinea pigs in this manner but using the same method they were also able to produce anaphylactic reaction in previously sensitized pigs.

The liver appears to exert a regulatory and detoxicating function during digestion. Presumably it removes undigested protein from the portal blood thereby interfering with its entrance into the systemic circulation. Egg white injected into the circulation appears in the urine sooner when introduced through

an ear vein than through a mesenteric vessel. When injected into a mesenteric vessel, it appears to be removed in part at least by the liver and make its appearance in the bile. In guinea pigs previously sensitized against egg white this protein causes death more rapidly and in small doses when injected directly into the systemic circulation than when introduced into the portal system.

Had investigations stopped at this point the current teachings of physiology would have required no alteration. Proteins are digested in the lumen of the gut and absorbed as amino acids except in the presence of local gastrointestinal disturbance with resulting increased permeability, when they may be absorbed incompletely digested, and produce sensitization.

But Rosenau⁵ succeeded in sensitizing guinea pigs following the *oral* administration of horse serum as did also Hettwer and Kriz. LaRoche, Richet and Saint Giron⁶ succeeded in sensitizing guinea pigs to egg white by the oral route. Stokvis⁷ as well as Van Alstyne⁸ showed that raw egg white taken into the alimentary tract may enter the circulation and be excreted through the urine. Finally Walzer⁹ and his collaborators demonstrated by the method of passive transfer that undigested proteins are absorbed and appear in the circulation of normal nonallergic individuals. A small amount of serum from an individual sensitive to egg was introduced into the skin of a nonallergic person. When subsequently the latter ate eggs, a local positive allergic reaction appeared at the site of the intradermal inoculation. The egg in the food was absorbed and carried through the blood to the site of the inoculation, its chemical makeup still sufficiently characteristic of egg white to give a specific response. This was demonstrated repeatedly not only with egg protein but also with fish protein.

Coca¹⁰ has shown that contrary to former belief, protein does pass in minute amounts through dialyzing membranes. If we consider the alimentary tract such a membrane he has shown that amounts of food protein sufficient to be of clinical significance may thus pass normally into the circulation. This observation explains those of Walzer and his collaborators.

So we must modify our concept to recognize that unaltered protein or only partially digested protein may normally be absorbed into the circulation. This does not clarify the question as to why some persons become sensitized while others do not, but it removes the site of initiation of sensitization from the intestinal mucosa to the tissues themselves. The localization of the allergic response in different organs or tissues remains unexplained.

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—W T V

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No 6

SYMPOSIUM ON CLINICAL BACTERIOLOGY

THE BROADER ASPECTS OF ROUTINE CULTURAL EXAMINATIONS*

BY RUTH GILBERT M D, ALBANY, N Y

THE subject is so comprehensive that the discussion is confined to a résumé of factors which tend to enhance the value of those aspects of bacteriologic procedures employed in examinations for physicians and sanitarians. Experience has proved that the work should be undertaken only in case it can be done expertly. The physician had better trust to his clinical findings and the sanitarian depend upon his inspection of environment or of methods and equipment than attempt to secure data from a laboratory unless practically implicit reliance can be placed upon its reports. The character, education, and training of the director and his assistants are thus of primary importance. The nature of the training which is required depends somewhat on the kind of work undertaken. If information is desired which would be of assistance in the diagnosis or treatment of individuals who are ill, the person in charge, besides having had adequate training and experience in pathology and bacteriology and in the fundamentals of chemistry, should be familiar with the clinical manifestations of disease. In case the laboratory serves the sanitarian also, its director should in addition have a knowledge of pertinent phases of sanitary engineering and the principles underlying the production of the materials to be tested. The technical assistants needed for all types of bacteriologic work should be thoroughly reliable and keen to note and report to their superior any unusual results which may be obtained. Their training should be adequate for the procedures assigned to them. Unsterile equipment and faulty technic may of course entirely vitiate the significance of bacteriologic findings.

A sufficient amount of satisfactory equipment is important. While an ingenious bacteriologist can often improvise suitable apparatus, his time and energy should be conserved for his professional duties if the supplies needed are purchasable.

*From the Division of Laboratories and Research, New York State Department of Health, Albany.

The procedures followed in bacteriologic examinations depend somewhat on the purpose for which they are undertaken, that is, whether they are designed to aid in diagnosis or to determine whether the patient is a carrier of a certain species of bacteria or can safely be released from quarantine. Another distinct group of examinations consists of those made for purposes of controlling sanitary conditions in the environment.

In case the physician wishes aid in diagnosis, the laboratory worker should, wherever possible, study the patient with the clinician and arrange for the collection of suitable material for examination. When this direct contact with the patient cannot occur, the physician submitting the specimen should furnish all the pertinent data which are available. The bacteriologist is then in a position to render intelligent service. When tests are undertaken to demonstrate evidence of the carrier condition or in connection with release from quarantine examinations of a routine nature are usually all that are required, although proper precautions must be taken to insure the collection of suitable specimens. Samples of water should be accompanied by information concerning the sanitary conditions which prevail in the environment of the sources, otherwise, the results of bacteriologic tests cannot be evaluated.

It is most desirable that samples of milk and water be examined in accordance with an established routine (standard methods), so that results from all laboratories may be comparable. Dairymen and other producers who have difficulties in maintaining their products at an adequate sanitary standard can frequently be given material assistance through the interpretation of some of these findings. The species of bacteria present often suggest their source and the points at which defects or faulty methods may be expected.

Specimens examined for purposes of diagnosis do not lend themselves to a definite routine. Each one must be considered individually, and only as every case becomes a research problem will the best type of service be rendered. In addition to the clinical manifestations the results of other tests or studies of his metabolism, tissue, blood, body fluids, or discharges must be considered. In fact, all of the data elicited during the examination of the patient should be correlated with the bacteriologic findings. At times, a simple morphologic examination of exudate may be sufficient to confirm the diagnosis, as in the case of gonorrhea and epidemic meningitis. More frequently, however, the bacteria seen in preparations from lesions do not have a sufficiently characteristic appearance to permit their identification, or microorganisms may not be found in such preparations. The history of the case may then provide a basis for determining the type of cultural study to be undertaken, the medium which will probably be most suitable, and whether aerobic or anaerobic conditions should be provided. When there is any uncertainty, experience has shown the desirability of inoculating sufficient medium to permit part of it being kept under aerobic, another portion under anaerobic conditions, and the remainder in an atmosphere containing from 5 to 10 per cent of carbon dioxide.

The microscopic examination of the original material, exudate for example, which is too often omitted, is essential to determine the relative number and kinds of bacteria, and finally to interpret the significance of the results of cultural study. The morphologic findings and knowledge of the possibilities for

contamination of the specimen during collection, indicate the amount of dilution required and the necessity for employing differential media or other means for the isolation of significant species which may be present

The time of incubation is most important. A long period may be necessary to secure growth. This is most frequently true in the case of highly parasitic species found in chronic infections, such as tuberculosis, certain streptococcus infections, etc. A month is none too long for the incubation of blood cultures, if growth is not secured earlier. Animal inoculation furnishes another means for determining the presence of some of the pathogenic bacteria. The species of animal chosen and the method of inoculation depend largely on the history of the case and the result of the morphologic examination of the specimen.

When cultures of microorganisms have been secured, identification usually requires a study of their morphology, biochemical reactions, behavior in sera from immunized animals, and of the type of lesion produced in inoculated animals. The evaluation of the findings may require a consideration of all the information secured in the study of the case. At times it may be desirable to determine whether the patient shows evidence of hypersensitivity to products of the bacteria which were isolated, or whether significant reactions can be secured with them in a specimen of his blood serum. The possibility of mixed infections must always be kept in mind. Either syphilis or tuberculosis, or both of these conditions, may of course complicate almost any other infection. When there is any doubt concerning the etiologic significance of the bacteriologic findings, specimens for confirmatory examination should, whenever possible, be secured.

To summarize, bacteriologic examinations made for purposes of conserving the public health, such as sanitary examinations of milk and water samples and those tests performed before release from quarantine or to determine the carrier condition, can well be conducted in accordance with standardized methods. Laboratory work which serves as an aid in diagnosis must, on the contrary, be undertaken, in most cases, as a special investigation, the procedures being chosen in the light of all the information available from a careful study of the patient.

USE OF ANIMALS IN ROUTINE DIAGNOSTIC WORK*

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LONG before the animate nature of disease was conclusively established Henle formulated a statement of the prerequisites which must be fulfilled in order to prove that a particular agent was the cause of an infective malady. He maintained that the agent must constantly be found in the disease. This constant agent must be isolated and tested in the isolated state to note whether it is capable of producing the disease. Obviously, this statement contains all the elements quite generally but incorrectly referred to as the "postulates of Koch (1878)." They serve as the unassailable basis upon which all the work on specific pathogenic causes is built. Despite the fact that subsequent observations placed certain restrictions and modifications on the original proposition, the testing of the isolated object or agent of disease must always remain a function of the bacteriologist. It is to be regretted greatly that the guiding propositions of Henle have frequently been overlooked in the routine diagnostic work of the modern laboratory. The reasons for this neglect are probably twofold. (1) Many of the workers who are called upon to do diagnostic work have had little or no experience in pathology. Even those who attend university courses have rarely had the opportunity to test the disease-producing properties of the common microorganism which they have isolated and painstakingly studied in the test tube. Lack of adequate funds, an unpardonable disinterestedness or one-sided training of the instructors may be mentioned as additional factors. (2) Though the importance of the pathogenicity tests in bacteriologic work is admitted until recently little attention has been paid to the animals which are required for the examinations. Only well-equipped diagnostic laboratories feel justified in maintaining an adequate stock of healthy animals. In some laboratories the number of rabbits and guinea pigs is so limited that each animal is a pet readily identified by an endearing name instead of a number. Others doubtless exist in which the animals are kept under unsanitary conditions and without proper care and feeding. Avitaminoses and chronic or intercurrent diseases are prevalent. It is needless to emphasize that costly, unpleasant surprises and many misleading observations are the obvious corollaries to such a state of affairs. Probably other criticisms could be offered. Suffice it to state that the prevailing conditions are largely the result of our own neglect. Until recently few publications paid attention to the "living test tubes" or the animals, largely because animal experimentation was considered the realm of the specialist and the study of the maladies of man a field which could not profit by an inquiry into the diseases of animals. More and more has this narrow conception vanished and the recognition that some communicable diseases of laboratory animals furnish excellent material for the

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study of fundamental problems in pathology promises an entirely new orientation

It is obviously not the purpose of this article to outline the various trends but merely to indicate the newer knowledge which the diagnostician must keep constantly in mind when he works with animals. In general the use of animals in the diagnostic laboratory is confined to the following procedures: (1) As a culture medium for the detection or rapid cultivation of various microorganisms (2) For the purification or elective isolation of certain types of bacteria from mixtures (3) For the propagation of virus diseases which cannot be maintained in artificial cultures (4) For the study of the pathogenicity or virulence and the pharmacologic and toxicologic effect of metabolic products (5) For the preparation of reagents, such as complement or diagnostic sera (6) To test the harmlessness or other effects of biologic products as well as their sterility, as in the case of filterable viruses

With unremitting insistence it must be emphasized that the execution of these procedures is successful only when certain fundamental principles are carefully considered and continuously carried out. The animals must be healthy preferably selected from a stock with a known hereditary history. They must be housed under sanitary conditions and fed an appropriate, well-balanced diet. The selection of an ideal place for the breeding of the required stock of guinea pigs and rabbits offers many technical difficulties. Adequate stable facilities, properly trained personnel and an abundant supply of inexpensive food are not usually at the disposal of the majority of diagnostic laboratories, or when available require an overhead expenditure which is entirely out of proportion to the number of animals required. For these reasons, the laboratories supply their demands by random purchases from dealers or breeders. The inherent disadvantages of such a policy are numerous. Rarely, if ever can the local market supply the varying demands for animals of different age, color, weight, sex, etc. Suitable animals in emergency for unexpected tests may therefore not always be in stock and the dealer supplies animals quickly assembled from various breeders. Such shipments may contain diseased animals. Until each city or state has a central animal supply station it is advisable to restrict the purchase of guinea pigs, rabbits and mice to that of contract from reliable dealers who raise their own stock and refuse to supply any other animals. Even these precautions fail only too frequently and expose the laboratory to the risk of introducing animals infected with a communicable disease. In order to reduce the danger it is imperative to hold each shipment in quarantine for at least two weeks. A special room should be reserved for this purpose and the animals should be housed in small groups. Preferably a single rabbit with pairs or lots of four guinea pigs may be placed in the same cage. The tag on the cage should carry a detailed history of the shipment. The results of the daily inspections should be recorded either on the tag or in a special book. Provocative tests for snuffles or stool examinations for *Salmonella* organisms and parasites should be carried out. Animals that die should be carefully autopsied and such laboratory examinations instituted as may be deemed necessary. The data should be submitted either to the chief animal caretaker or to the person responsible for the animal quarters. In case an infectious

disease is discovered it is much more economical to use the shipment for sacrifice experiments than to prolong the quarantine and trust to luck that the malady will not spread. Latent infections with the B. cuniculi-septicus are frequently activated by the injection of biologic products and thus the experimental animals may act as spreaders of the disease. It appears unnecessary to emphasize that the caretaker in charge of the quarantine room should exercise special care in the disinfection of the feeding cups, cages, etc., and be instructed in methods to break the chain of transmission through utensils, hands, or clothing, from the quarantined stock to the experimental animals. While in quarantine the animals are freed from vermin and ectoparasites. These things appear self-explanatory but they are often overlooked.

The suggestion that a group of laboratories in a state or city pool their resources and encourage an interested party to breed laboratory animals for certain purposes deserves serious consideration. More and more it becomes evident that the fundamental laws of infection must be studied on litter mates or preferably on animals with a known hereditary history. The brilliant researches of Maude Slye on the transmissibility of tumors, those of Lewis and Wright on the varying susceptibility of guinea pigs to tuberculosis, and those of Webster and Pritchett on the inborn, nonspecific resistance of mice against B. aertrycke infection offer ample proof that in the future even diagnostic studies must be conducted on animal species with a known inherent susceptibility to certain infections. Provided the animal breeding institute is under the supervision of a geneticist, an abundance of invaluable information can be collected and subsequently correlated with the findings of the biologist and pathologist. Thus, the need for special, short-haired rabbits or albinos for skin tests could be met with ease, and litter experiments would be more frequently conducted than has hitherto been the case.

Those who desire to raise a limited stock of mice or guinea pigs should familiarize themselves with the behavior, breeding habits, and gestation periods. Excellent, thoroughly scientific summaries on the mouse, rat, guinea pig and rabbit are available in book form (Keeler, Donaldson, Raebiger) or in a chapter of the *Handbuch der pathogenen Microorganismen* and in *A System of Bacteriology in Relation to Medicine*.

There is an increasing tendency to standardize the *cage equipment* in the different laboratories. Excellent designs, although expensive, are now manufactured by several companies. Metal cages are quite generally used for individual experimental animals, while wooden runs or entire rooms with wooden floors are preferred for stock guinea pigs and rabbits. However, it is well to remember that even the best cages will not prevent disappointment when their care is superficial and sporadic. It appears unnecessary to outline the essentials since Wadsworth has admirably summarized the rules which should be followed. Cages or jars which house animals with highly communicable diseases should be kept in special rooms and should only be handled by the worker in charge of the respective tests. In fact, he or she should be directly responsible for the feeding of the animals while in use, and the disinfection and cleaning of the containers after they are vacated. It is doubtless an advantage to tag such cases or jars with specially colored labels. Many laboratories use sawdust as

a bedding Experience has taught that shavings are preferable, particularly for rats It is not unlikely that the troublesome lung disease of these animals is greatly augmented by improper bedding

The animal quarters should be well lighted and ventilated A uniform temperature during the entire day and night should be provided Special attention must be given to the feeding of the animals Only too often this phase of the animal experiment is sadly neglected Although excellent diet tests have been reported, it appears desirable for quick reference to summarize the pertinent data with respect to each species of animals

1 *Mice* may receive stale bread soaked in water or skimmed milk A small amount of cod liver oil may be added once a week (approximately one ounce for 300 mice) Crushed barley or rolled oats or moistened middling may be offered at regular intervals An excellent ration recommended by Keeler consists of rolled oats (240 parts), powdered skim milk (30 parts), cod liver oil (8 parts) and salt (one part) The formula for rat feed prepared by Maynard (*Science* 71 192, 1930) is equally satisfactory Greens in the form of lettuce or clover should be given occasionally Drinking water must be available always

2 *Rats*—One can either use the Maynard standard diet or prepare a mash which consists of boiled beans, wheat maize meal, cabbage and cod liver oil Beef or liver and fresh cabbage should be offered once a week A mixture of boiled vegetables supplemented by oats corn, or white bread mixed with milk is equally satisfactory Drinking water should always be available

3 *Guinea Pigs and Rabbits*—Rolled oats or crushed barley, bran, and a good quality of alfalfa or clover may represent the basic ration which must be supplemented with green feed, either cabbage or carrots, and beets From time to time salt, fish meal, and boiled potatoes may be offered If the food contains considerable moisture no water need be supplied Spontaneous scurvy is by no means uncommon among guinea pigs maintained on a diet which lacks green stuffs (Smith, Holst and Frolich, Funk)

Aside from a careful selection of freshly prepared food mixtures or wholesome vegetables and greens freed from tainted or rotten spots, it is important that a regular system of feeding be strictly adhered to All animals should be fed and cared for once a day including Sunday A double ration of food thrown into the cages on Saturday will not supply the required nourishment on Sunday

A dependable system of identification of the animals should be adopted The practice of cage labeling is suitable for stock animals kept in large groups although in the interest of complete records each animal should be numbered and registered with regard to sex, description etc Guinea pigs and rabbits may be identified readily with the aid of aluminum ear tags (Aluminum Marker Works Beaver Falls Pa) or India ink ear marking sometimes supplemented by a diagrammatic notation in the record book while rats and mice are marked either by puncturing the ears a system introduced by the American geneticists or by color markings with aniline dyes In the diagnostic laboratory, the latter procedure recommends itself on account of its simplicity

The worker who uses animals should be thoroughly familiar with the normal anatomy and physiology of the guinea pig rabbit rat and mouse He

should have at his disposal such standard works as W. Schauder, *Anatomie der Impfsaugetiere* and in particular the monumental treatise by Rudolf Jaffe, *Anatomie und Pathologie der Spontanerkrankungen der Kleinen Laboratoriumstiere* and the *Tabulae biologicae*. Valuable information will also be found in the well-known treatises on the rabbit by Bensley and on the rat by Hunt.

Systematic postmortem examinations of every animal which is found dead in the animal house or those which are sacrificed for various reasons offer an inexhaustible source of information and training. Freedom from epidemic or parasitic diseases which, when not recognized, may seriously invalidate an animal test, largely depends upon these thorough and conscientious anatomic examinations, as well as upon an appreciation of the epizootiology of the infections and their effective control by quarantine and the other preventive measures. Without a fundamental knowledge of the normal and pathologic anatomy of the common laboratory animals the diagnostician and research worker fail to appreciate the limitation of the animal tests. The significance of spontaneous infections and parasitic invasions in the animal stock is now fully recognized and in addition to the book by Jaffe, several articles (K. F. Meyer, Raebiger and Lerche, Remlinger and P. Bell, O. Seifried, Gerlach, Farmer's Bulletin No. 1568) have detailed the most important facts. It is not within the scope of this review to attempt even a brief summary of the many maladies which may be encountered. An opportunity will be afforded to illustrate the importance of the intercurrent disease as factors in the diagnostic animal test.

Every modern textbook of bacteriologic technique (Kolmer and Boerner, Smith, Eyre, Wadsworth, Haberland, etc.) contains excellent commentaries on the methods of manipulation, inoculation and bleeding of animals, but one essential principle is rarely stressed. Immediately upon the inoculation of animals a period of clinical observation begins which should terminate only with the death of the animal. These observations should always take cognizance of

(a) *The General Appearance*—Inspection once or preferably twice daily should be given with a view to detect symptoms. Each animal should be observed in motion, the consumption of food and water and the appearance of the

TABLE I
NORMAL TEMPERATURE, PULSE, AND RESPIRATION OF EXPERIMENTAL ANIMALS

ANIMAL	AVERAGE RECTAL TEMPERATURE °C AND °F	PULSE RATE	RESPIRATION PER MINUTE
Guinea pig	38.6° C, 39.4° C—7.5 cm. from anus (101.5° F—4 cm. from anus) (Minimum 37.8° C, maximum 40.5° C)	150	100-150
Rabbit	39.6° C (102.4° F) (Minimum 38.3° C, maximum 40.8° C) No temperature under 40.0° C should be considered pathologic	120-140	50-60
Rat	37.9° C (100.0° F)	—	210
Mouse	37.4° C (99.3° F)	120	—

feces should be noted. Attention should also be paid to possible salivation, nasal and conjunctival discharges, and to the reactions at the site of inoculation.

(b) The *weight* should be recorded before experiment and afterwards at weekly intervals or more often as the circumstances require. Each weighing should be done as nearly as possible under the same conditions as the first one.

(c) The *temperature* should be taken in many cases before beginning the particular experiment and subsequently on each successive day at the same hour. For the sake of convenient reference the normal average temperature is given in Table I in order to prevent the erroneous assumption that a pyrexia is present in an animal which shows merely its own normal temperature.

(d) A *hematologic study* is frequently indicated. The general principles are the same as customarily used in the diagnostic laboratory. The normal averages of the different blood cells are summarized in the book by Kheneberger and W. Carl, and in the chapters by E. Haam and E. Flaum in the *Handbuch* by Jaffe (p. 155).

(e) The *bacteriologic, serologic, and autopsy* examinations differ in no way from those generally employed.

Provided the principles briefly outlined are followed the diagnostician should experience no disappointment in the use of the animal test. Since a perusal of the usual text fails, however, to mention the type of animal most suited for certain examinations, it is deemed advisable to offer certain suggestions. Thus, an opportunity will be afforded to indicate the complications which may arise should the mice, guinea pig, or rabbit be spontaneously diseased. For the sake of convenience, the subject is discussed under the heading of the respective infections.

1 *Staphylococcus Infections of Intoxications*—The pathogenicity of a staphylococcus should be studied on the rabbit or on Japanese white mice. Significant lesions may be observed following intravenous injections, while local reactions may be induced by scarification of the cornea. Toxic metabolic products may be tested by intravenous injection (Dack and associates) or by intradermal application (Parker, 1924).

2 *Streptococci*—There is some difference of opinion as to which animal is most susceptible. If relative sizes of animals are considered the rabbit is the most readily attacked. Various methods of infection are chosen in order to imitate the pathologic process from which the coccus has been isolated. Virulence tests are frequently made on mice. Great care should be exercised in the interpretations of the bacteriologic findings on these animals since several workers (Zlatogoroff, Palanto and Kochkina, Grossmann and others) have found that hemolytic and nonhemolytic streptococci have been isolated from supposedly "normal mice." Therefore the supposed mutation of pneumococci and non-hemolytic varieties by intraperitoneal injections (Morgenroth, Schnitzer and Berger) deserves further investigation. Latent infections due to hemolytic streptococci are quite common in guinea pigs. They frequently invalidate the diagnostic experiments.

3 *Pneumococcus Infections*—The extreme susceptibility of the mouse to the pneumococcus is a commonplace of laboratory experience and forms the

basis for the rapid isolation of the organism from bacterial mixtures, sputum, etc. Although the mouse may be killed by a smaller dose than the rabbit, it is generally recognized through the comparative studies of Cafeiro, Cotoni, Truche and Raphael that the rabbit is more susceptible. In an emergency this animal can therefore be used for the typing of pneumococcus strains. Experimentation on guinea pigs requires careful and critical interpretations since spontaneous pneumococcal infections may be unusually common in the stock of certain breeders.

4 *Influenza Bacilli*—It is well to remember that autolyzed influenza bacilli may set up spontaneous streptococcal or pneumococcal infection in guinea pigs and mice. A general blood infection may be produced regularly when the sputum to be tested contains the symbiotic adjuvants, the cocci (Wolf, 1920, Huntoon and Hammin).

5 *Tuberculosis*—The detection of tubercle bacilli by animal inoculation offers several disadvantages. Not infrequently this species suffers from chronic diseases such as pseudotuberculosis or Salmonella infections which present gross anatomic lesions readily confused with those of tuberculosis. Spontaneous tuberculous infections of the guinea pig and rabbit have been reported by Feyerabend, Seifert, Sewall and Lurie, Stanley, Griffith and others, and thus may greatly invalidate the significance of the test. In part, these disadvantages may be overcome by a judicious interpretation of the postmortem findings. It is a well-known fact that in susceptible animals the primary localization of the lesion always indicates the avenue of infection. If, for example, after subcutaneous inoculation a tuberculosis of the tracheobronchial lymph nodes with no disease in the inguinal nodes is found, one may conclude that the infection was acquired from extraneous sources.

6 *Plague*—*B. pseudotuberculosis rodentium* (Pfeiffer) occurs spontaneously in guinea pigs and may thus interfere with the diagnosis of plague (Indian Plague Commission, J Hyg 7 256, 1907).

7 *Brucella Infections*—It is not generally appreciated that isolated cases of melitensis (Nicolle and Conseil) and abortus infections (Surface) have been reported in animals. A extensive epizootic which was caused by a melitensis type and affected 400 guinea pigs has been reported by Zdrodowski (1927 and 1930). In view of the wide distribution of the Brucella organisms, it is imperative that in the future shipments of guinea pigs from unknown breeders be scrutinized for Brucella infection. Agglutination tests previous to the inoculation of the test material are exceedingly useful.

8 *Anaerobic Infections*—Those who use the guinea pigs or rabbits for the primary isolation of a pathogenic anaerobe should always remember that certain species may be found as common parasites in the intestinal tube or even in the organs of these animals (Heller, E. Schmidt).

9 *Paratyphoid Infections*—The fact that *B. aertrycke* orally administered to mice sets up a lethal septicemia, while *B. paratyphosus B* (Schotmuller) usually does not, is sometimes used as a practical differential test of these two organisms. Unfortunately, its value is greatly reduced on account of the widespread occurrence of latent aertrycke infection in the rodents. Experimentation

with paratyphoid bacilli on mice requires sound judgment. The bacteriologic literature reports such bizarre findings as the transformation of a B paratyphosus into a B enteritidis or B aertrycke.

10 *Virus Infections*—The controversial literature on experimental encephalitis clearly shows that latent widespread parasitic infections may be responsible for misleading conclusions. Furthermore, the discovery of a new virus by Miller, Andrewes and Swift, in the course of an attempt to reproduce experimental rheumatic fever, furnishes another example of the many obstacles which may continue to render the animal test a very complicated diagnostic procedure.

Many more observations could be cited. In particular, attention might be drawn to the deficiency of the hemolytic complement in the blood of certain races of guinea pigs owing to the absence of the third component (Hyde). Suffice it to emphasize that the bacteriologist who employs the animal as a test object or model in his diagnostic work should be in a position to defend his claims that the lesions or findings are not those of a spontaneous disease but the result of the experiment. This aim can be met to a great extent if he employs only well-bred animals with a known hereditary history free from bacterial infections and parasitic invasions, maintained and cared for by an experienced personnel and kept on well-balanced diets in a sanitary and hygienically controlled environment.

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ANAEROBIC TECHNIC^{*}

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ATTENTION was directed by Pasteur (1859-61), during his investigations dealing with the so-called "butyric ferment," to the existence of micro-organisms capable of living and multiplying in the absence of oxygen. He subsequently introduced the terms "aerobe" and "anaerobe" to indicate and emphasize the gaseous requirements of microbial life. As a result of the publications of Koch on anthrax, Pasteur became interested in this disease. With his associate Joubert, suspected anthrax blood that had undergone putrefaction was studied and from this material an anaerobe, the vibron septique, was isolated. Experiments demonstrated the new germ to be pathogenic for animals. Quite naturally this work stimulated the search for other germs which bore this peculiar relationship to air, with the result that several anaerobic organisms were early detected and technical methods for their cultivation were developed and employed.

From the time of Pasteur's discovery down to the present day there has been one continuous stream of anaerobic apparatus of every size, shape, design and material, all based on permutations and combinations of a few elementary principles, namely, exclusion of air, exhaustion of air, absorption of oxygen, replacement of air with an inert gas, apparent presence of air, microbial or tissue association. It is characteristic that each device or procedure is usually referred to as new and simple.

The anaerobic bacteria have ordinarily constituted a class by themselves set apart from the usual routine in bacteriology, unquestionably because of the supposed difficulties of a technical nature attending their investigation. The problems surrounding their study have been exaggerated and overemphasized (perhaps unintentionally) by teachers, to the extent that there has been instilled in the mind of the ordinary laboratory worker the feeling that anaerobic life is a field of investigation reserved for those specially trained workers of which each nation had but few, with an unlimited supply of fanciful equipment. In fact, during the past only a very few laboratory courses in bacteriology included actual experiments on the isolation and identification of anaerobes. That this class of organisms was of great economic importance was well known, not only were they associated with a number of diseases of man and animals but it was understood that putrefaction or at least the initiation of putrefaction was a process which these bacteria carried on almost alone.

The World War focused attention on anaerobic technique primarily because of the serious consequences resulting from the multiplication of these organisms in wounds with their sequel of gas gangrene. It is true that such infections had commanded attention before but the incidence was rather infrequent.

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Commissions were appointed to search the literature for practical procedures, to develop methods if such were not in existence and to test thoroughly all recommendations under routine laboratory conditions, with the end in view of making available a simple satisfactory technique for the study of this group. Adequate directions were found, which, if followed, gave excellent results in the hands of those willing to employ the precautions essential to insure the complete absence of air during the incubation period of the culture, as well as a minimum exposure of the germs to air at all times. It is deserving of mention that the quantity of oxygen which inhibits the growth of anaerobes is small.

The concentration by many workers on the flora of war infections resulted in the addition of several members to this group, and also brought together, in an easily available form the literature relevant to the subject. But no striking technical procedures or new pieces of apparatus were evolved which demonstrated marked superiority to the methods of twenty years before. It is characteristic of the postwar literature, however, to refer to "the chaotic state of the pre-war anaerobic knowledge and the fundamental advances due to improved methods of anaerobic cultivation developed during the war period." Perhaps the most tangible contributions came in the pointing out of the necessity for a familiarity on the part of all bacteriologists with methods of pure culture study, that anaerobic organisms could be streaked on the surface of solid media preferably blood agar, in Petri dishes, surface colonies obtained and studied with the subsequent selection and inoculation of differential media in the ordinary procedure of identification. Above all that a bacteriologic study of unknown material was not complete until the question of the presence of anaerobes had been satisfactorily disposed of. Nevertheless it is a matter of common occurrence to find that the knowledge reawakened by the war studies has lapsed and today the average complete bacteriologic examination of suspected materials consists of the isolation and attempted identification of the aerobic flora.

The methods to be presented in the following pages are largely based on those used by Professor Novy (1893) in his studies, particularly with reference to the isolation of *B. novy*. Naturally, since that time many important improvements in technique have been made and such aids have been incorporated in the general procedures as soon as their practical value was demonstrated.

MEDIA AND APPARATUS

It is essential in anaerobic procedures as well as in studies dealing with aerobes to be able to isolate the organisms in pure form and subsequently cultivate them serially upon suitable media, for only in this way is a knowledge of their function possible. A uniform set of cultural tests is of great advantage in distinguishing the various species and for this purpose the following media have been found to possess distinct value.

Litmus Milk.—To fresh skim milk is added a very concentrated aqueous solution of purified blue litmus sufficient to impart a decided blue color. After thorough mixing it is placed in test tubes to a depth of two or three inches and sterilized in the Arnold sterilizer for thirty minutes on each of three successive days. It can be sterilized in the autoclave at 110° C. for ten minutes.

Too much heat should be avoided, since it may cause an alteration of the milk. Milk is difficult to sterilize because of the presence of thermophilic organisms. Hence, it is advisable to place the tubes containing the milk at 37°C for several days to make sure of its sterility. Litmus milk is used to ascertain whether an organism can coagulate casein, and whether it can decompose the lactose, thus giving rise to gas bubbles or to acid products. The medium when correctly prepared should have a lavender color, which turns red in the presence of acid and blue when bases predominate.

Coagulated Serum—The usual horse or beef serum medium coagulated on the slant in test tubes is used to determine the liquefying power of the cultures.

Litmus Gelatin—The ordinary 10 per cent nutrient gelatin containing purified litmus is used. Certain species liquefy gelatin and the litmus acts as an indicator of acid or alkali production and in addition it may be reduced.

Blood Agar Plates—The ordinary beef infusion agar is used as a base. The agar is melted, cooled to 50°C , and 20 per cent of defibrinated blood (horse, cow, human, or rabbit) added. The medium is then thoroughly mixed and poured into Petri dishes under aseptic conditions, and allowed to cool. A dry surface is essential to avoid a confluent growth. Well-isolated colonies are obtained in from twenty-four to forty-eight hours when a droplet of inoculum is placed on the surface of the medium, spread with a bent glass rod and incubated under anaerobic conditions.

Cooked Meat—Carefully trimmed heart muscle* (beef) is finely comminuted, added to 2 parts of distilled water and stored in the ice box overnight. One per cent peptone and 0.5 per cent salt are added and the infusion is heated in the boiling water-bath for one hour. The water lost by evaporation is replaced and the reaction adjusted to $\text{pH } 7.4$ with $\text{N}/1 \text{ NaOH}$. The medium tissue as well as extract is placed in test tubes and sterilized by the fractional method for thirty minutes on each of five successive days. The tubes which contain some 2 inches of cardiac muscle overlaid with about half an inch of broth are incubated several days to insure sterility. Sterilization may be carried out in the autoclave if care is exercised to avoid the blowing out and spattering of the cotton plugs by the tissue. This is a very valuable medium for the cultivation and identification of anaerobes. The tissue supplies useful nutritive substances to the medium and it also acts as a reducing agent and thus removes any dissolved and toxic oxygen. Characteristic changes in reaction, color of the meat, and varying degrees of digestion also occur which aid in the differentiating of the members of this group.

Dextrose Agar, Dextrose Broth—The usual beef infusion media containing 2 per cent dextrose is used. The former is very useful for plates, shakes and stabs. The broth is used for making dilutions and on the addition of 5 per cent sterile blood is valuable for growing anaerobes direct from the animal body.

The above media when used in test tubes are placed just before inoculation in the boiling water bath for five or ten minutes to drive out the dissolved air and are then cooled to 40°C .

* Certain workers prefer to use a medium containing brain tissue.

The pipette of the Pasteur School is to be preferred to the platinum loop for the inoculation and transferring of the cultures. The pipette is employed to transfer liquid cultures, pus, blood, transudates, etc., or surface growths from solid media. In the latter case a drop or two of sterile broth is mixed with the colony or growth, and a fine suspension made and drawn up into the pipette. It is a common experience that larger quantities of anaerobic material must be transferred to get a good growth of organisms than are necessary when working with aerobes.

The inoculum is always discharged into the bottom of the medium in the tubes. No overlaying seal to keep out the air is absolutely necessary, since reducing substances are present in the milk, gelatin, and meat media. Sterile yellow petrolatum to a depth of about one centimeter is in quite general use and forms a very excellent seal when placed on the top of these media. In addition to the exclusion of air, it prevents desiccation. The presence of the seal does not seriously interfere with the inoculation or withdrawal of medium, as the end of the pipette will readily penetrate the petrolatum. The dextrose agar tubes are usually thoroughly mixed after inoculation and used for shake cultures.

Culture Tubes—The plain test tubes (15 by 150 mm.) of resistance glass are employed. They are thoroughly cleaned, plugged with cotton, and sterilized in the dry heat oven before the introduction of the medium.

Jars for Plate Cultivation—The Novy jar,* with the special cock for vacuum work, is to be preferred. The following directions apply to this apparatus, vacuum desiccators and museum jars have been used and found satisfactory, but not as convenient as the specially designed jar.

The lower half of the jar should have an internal diameter sufficient to allow the easy introduction and withdrawal of the ordinary 100 mm. Petri dishes. Jars of this type are made in two sizes, one with an internal diameter of 13 cm. and an internal height of 12 cm., the other has the same diameter with a height of 20 cm. The shallow jars will hold from six to eight Petri dishes and the deep jars will accommodate from twelve to fifteen plates. These jars are also used when large numbers of tube cultures are to be incubated at one time. The actual procedure is as follows. The surface of the medium, blood agar or glucose agar, is inoculated with a droplet of material from a pipette (poured dilution plates are frequently made). The inoculum is then spread, using a sterile bent glass rod. The plates are stacked in the lower portion of the jar and on top of the pile of plates is placed one of the halves of a Petri dish containing ten or fifteen thin slices of freshly cut raw potato, prepared with no attempt at sterility. The respiration of the potato, when the cover is placed on the jar, supplemented by that of aerobic organisms present will remove the traces of residual oxygen in the confined space and growth of the spores and vegetating forms will take place. There are objections to the use of excessive amounts of raw potato in the jars. Water is a respiratory product and the confined atmosphere, after a time, becomes supersaturated,

*Novy jars unless made according to the original specifications are unsatisfactory. They can be obtained from Greiner and Friedrichs Stützerbach im Thüringen, Germany.

with the result that the condensation of moisture takes place on the agar surfaces thus favoring a confluent growth rather than the desired isolated colonies. In addition, there is ample proof that a definite tension of carbon dioxide favors the growth of aerobes and anaerobes alike, but excessive concentrations of this respiratory product serve as an inhibitory factor because of the acid nature of the substance in solution. The flanges of the jar are lightly greased with *lubri-seal* the cover put in place a rubber band (16 by 130 mm) is applied to the circumference, and six or eight small vises are then carefully and evenly tightened to the flanges. The main stopper should be wired in place. The jar can be made air-tight with very little care. The objection usually directed at the Novy jars is the difficulty of opening them after they have been in the hot room for several days. It is possible to loosen the top piece with an eccentric lever which is applied to the upper flange under moderate sustained pressure. The jars can be easily opened and breakage rarely occurs when warm water is applied to the flanges.

After the jar is closed and sealed, the glass cock is connected to a source of carefully washed hydrogen. This gas is best prepared in a Kipp generator, using dilute H_2SO_4 and granular zinc. The hydrogen so generated is passed through a series of four wash bottles containing alkaline lead acetate 5 per cent solution of potassium permanganate, 5 per cent solution of silver nitrate, alkaline pyrogallate, and then into the culture jar. The gas must flow at a slow rate so that all impurities will be scrubbed out.

A vacuum line with a manometer and stopcock is joined to one end of the special stopcock on the jar. The purified gas is led through an auxiliary stopcock into the other end of the special stopcock on the jar. To fill the jar the gas supply is turned off and the vacuum line is opened, a vacuum of about 600 mm is quickly drawn on the jar the stopcock on the vacuum line is closed, and gas is led slowly into the jar through the open stopcock on the gas line until the negative pressure in the jar reaches zero. This latter stopcock is now closed and a vacuum again drawn in the jar with subsequent refilling with the washed gas. This procedure is repeated five times at the conclusion of which only traces of free oxygen are present in the atmosphere of the jar. The jar is sealed by turning the stopper and then placed in the incubator. The small amount of oxygen still present will quickly disappear due to the respiration of the potato. After an incubation period of from two to four days the jars are opened and the plates examined. Well isolated colonies are selected and transferred with a pipette to the several media.

The catalyst principle for the removal of oxygen from the container or jar was introduced during the war and it has found considerable favor with a number of workers. The active unit is ordinarily composed of asbestos impregnated with palladium or platinum and is suspended from the cover of the jar with suitable wire connections for attachment to the source of electricity which is used to activate the catalyst when the jar is closed. The inoculated plates or tubes are placed in the jar the cover is attached and the confined atmosphere is replaced with hydrogen. The electricity is turned on to heat the catalyst and the residual oxygen combines with the hydrogen under these conditions.

The heat is usually continued for at least half an hour and then the wires are disconnected and the jar is placed in the incubator.

Unsatisfactory results have been experienced by various investigators with the inert gases used to displace the air in the jars. Hydrogen as obtained from the electrolysis of water, and nitrogen, a by-product of the liquid air industry are available commercially under pressure in large drums. The ease of handling the gases in these containers and the necessity for the constant refilling of the Kipp generators when large volumes of hydrogen are used quite naturally encouraged many workers to secure these gases direct from the tanks and lead them without purification into the jars to displace the air. The unfavorable growths obtained when these tank gases have been used were unquestionably due to the presence of oxygen. It is the exception to find on analysis an oxygen content of less than 2 per cent in ordinary tank nitrogen. Samples from several tanks have had an oxygen concentration as great as 6 per cent and the ordinary tank hydrogen assays only a little better as far as oxygen content is concerned. Tank gases should be passed through an oxygen absorbent before they are led into the jars, or larger quantities of raw potato must be placed in the jars to take up the undesirable oxygen introduced. Traces of the lubricating oils used on the compression machines are also present in the tank gases and should be removed along with the oxygen.

GENERAL PROCEDURE

The exact procedure to be followed in the determination of the anaerobic flora of infected material depends quite naturally on the data desired and the substance under investigation. For example, when the presence or absence of one particular germ is routinely desired in a certain substance such as B. welchii in fecal material, a procedure limited to the detection of this organism is usually followed. On the other hand, when the isolation and identification of the complete anaerobic flora are demanded, a technique must be employed which will take into consideration the nature of these organisms and the various interfering and inhibiting influences.

Infected material containing a pure culture of only one organism is the exception rather than the rule, and materials to be examined for anaerobes are usually rich in aerobes. Many difficulties attendant to the isolation of anaerobes from cultures containing aerobes as well as the separation of an anaerobe from a mixture of anaerobes and the various possible combinations must be given consideration. Over thirty pathogenic and forty saprophytic species of anaerobes have been described and almost every member of this group, singly or in combination with others, has been isolated from a wound infection at some time or other.

Consideration should be given to the following possibilities:

Sporulating and Nonsporulating Anaerobes—Blood from human and animal sources and tissues from artificially infected animals usually come under this heading.

Sporulating Anaerobes With Nonsporulating Aerobes—These are found in infected muscle removed at operation, pus from wounds, postmortem substances—

in brief in material of human and animal origin. As a rule such material is rather simple to handle. If the preliminary microscopic examination demonstrates the presence of spores selective heating at 80° for twenty minutes is sufficient to remove such forms as the staphylococci, streptococci, *B. proteus*, *B. proteus* and the coli forms.

Sporulating Anaerobes With Sporulating Aerobes—Specimens of soil, water, milk, milk products, and the various pickled and preserved foods generally contain sporulating aerobes. Most of the aerobic spore formers grow very slowly under anaerobic conditions.

Nonsporulating Anaerobes With Sporulating and Nonsporulating Aerobes—Naturally this combination is more difficult of separation. Heating is obviously out of the question. Primary enrichment (presented later) followed by surface cultivation with the picking and subculturing of a considerable number of the several varieties of colonies obtained ultimately yields pure strains. The procedure is time-consuming, tedious, and often disappointing.

A sample sufficiently large to inoculate several tubes of medium should be taken. Samples of food products if distinctly acid are neutralized before inoculating, the same for specimens of soil.

Before proceeding to the microscopic or cultural studies a general examination of the suspected material should be made. The origin of the specimen and its color and odor should be noted. A cover slip or hanging drop preparation is made. In the case of solid materials, such as necrotic tissue, the material is touched several times to a droplet of water on a glass slide, a cover slip is subsequently dropped onto the preparation, and it is ready for the microscopic examination. Semisolid substances such as pus, feces, and sputum are first emulsified with several drops of water while clear fluids such as urine, spinal fluid, etc., are centrifuged and then a portion of the precipitate submitted to direct examination. Care should be exercised especially in noting the form and grouping of the cells, the presence or absence of motility, the appearance of the protoplasm, and the presence of spores. While it is well known that abnormal forms frequently occur and that in the animal body departures from the typical morphology are frequently found, it is true that the information gained in this study may point the way later to forms that might be overlooked.

Methylene blue and Gram stains should be made of fixed films. The choice of Gram technic depends upon the nature of the material. It has been our experience that if one masters and routinely follows one procedure for this differential stain rather than changing the method to suit the material, the results are of greater value. Large gram-positive rods in pathologic materials are apt to be anaerobic bacilli. At best the results obtained from the preliminary direct and tinctorial examinations are merely suggestive.

Quite naturally the primary aim is to preserve all the anaerobes present in the sample taken, and for this a medium as little selective as possible should be used. The dextrose litmus gelatin medium was found for a long time to be very useful for this purpose but it was ultimately replaced with the cooked meat medium. It has been pointed out that certain organisms such as *B. welchii*, *B. fallax*, and *B. aerofetidus* tend to die out rather rapidly in a

dextrose-containing medium The cooked meat medium is not only of importance because of its nutritive value and stimulating action on the growth of all germs but, in addition, the content of buffer substances makes it most suitable for the preservation of the microbial flora, even the less resistant forms will remain alive in this medium for years, so that one may return as often as desirable for subcultures

Two tubes of the cooked meat medium are heated in the boiling water-bath for five minutes and then cooled to 40° C A sample of the unknown is transferred to each of the tubes and the contents are then thoroughly mixed The medium is then overlaid with sterile petrolatum One tube is placed immediately at 37° C, the other tube is heated to 80° C for twenty minutes and then placed at 37° C The former tube gives an index of the anaerobic and facultative anaerobic flora and the heated tube will account for the sporulating forms

Observations are made at frequent intervals during the incubation period for evidence of growth, such as gas formation, color, and possible digestion of the meat tissue The richness of growth will depend upon the original inoculum

At the end of twenty-four hours hanging drop preparations and stains are made from both tubes If growth is absent, incubation is continued for at least two weeks before discarding the tubes

If growth is present, glucose agar shake tubes are prepared Tubes of glucose agar are melted and cooled to 45° C, a drop or two (depending on richness) of the culture is transferred to a tube of the melted agar and thoroughly mixed From this tube two serial dilutions are made in the same medium and after thorough agitation the medium is permitted to solidify Half-inch seals of sterile agar are superimposed on the medium and the tubes are incubated at 37° C

Three blood agar plates are inoculated with droplets of the culture from each of the original tubes and quickly spread with a sterile bent glass rod the amount of inoculum transferred again depending on the richness of the inoculum The six plates are placed in a jar, the jar closed and at once subjected to anaerobic conditions, and then placed in the incubator

Incubation of the two original inoculated tubes of meat medium is continued with frequent observations It has been found that if a mixed growth of anaerobes occurs in a tube of meat medium the germs first to develop are *B welchii*, vibrios septique, and *B fallax* This takes place within the first twenty-four hours At the end of forty-eight hours' incubation the predominating organism will be *B sporogenes* and a third phase of growth occurs at the end of ninety-six hours when *B tetani* predominates Thus, agar shakes and plate cultures made at these three intervals are most likely to give satisfactory isolations

After forty-eight hours' incubation the plates are removed from the jars and the surface colonies examined microscopically with the low power lens It is extremely important not to rely on the unaided eye in a consideration of colonies One which appears to be well isolated macroscopically, may, on examination with the lens, be surrounded by many others or it may be seen to have de-

veloped in the midst of a surface film. After hanging drop examination and stained preparations have been made, well-isolated colonies of each variety present are transferred with a pipette to selective media, such as cooked meat, litmus gelatin, litmus milk, coagulated serum, and agar slants. These subcultures are incubated and examined at regular intervals and the changes are recorded. It should be emphasized that changes in the media go on for a much longer period in the case of anaerobes, their full extent cannot be expected under ten or fifteen days. It is extremely important to control every anaerobic isolation for the presence of aerobes by inoculating an agar slant and incubating it under ordinary aerobic conditions.

The colonies in the agar shake tubes are examined with the hand lens and if found to be well separated, several of each type, if more than one variety is present, are selected, a slight scratch in the glass is made with a file directly over the colony and the edge of the scratch is touched with a hot glass rod thus causing the tube to crack, thereby exposing the medium which is placed in a sterile Petri dish. A sterile knife is used to slice the cylinder of medium in close proximity to the colony. Tubes of selective medium are inoculated from the colony and on incubation one rarely fails to secure a pure culture. It is probably true that this method gives the most consistent results in the hands of the beginner who is usually unappreciative of the strict requirements of the plate method.

The criterion for purity is the consistent behavior of a culture when submitted to growth on different media over a long period of time.

IDENTIFICATION

Identification may frequently be anticipated from the origin and nature of the specimen as well as from the data accumulated during the isolation. Short cuts are often resorted to such as the direct inoculation of milk with a bit of the original material. The occurrence of a characteristic stormy fermentation in from twelve to twenty-four hours is diagnostic of *B. welchii*. It is advisable, however, to carry through the routine procedure. The characteristics available as a basis for identification can be grouped under the following categories: morphology, cultural, biochemical reaction, serologic reaction, the production of characteristic toxins and pathogenicity. No one of the above aspects taken by itself furnishes sufficient data for a satisfactory identification. Thus a combination of these various attributes has to be relied upon.

Information regarding the size, shape and structure of the cells is usually obtained from an examination of the purified strain in the hanging drop as well as in stained specimens. Considerable care must be exercised in the judgment of motility. A direct drop quickly covered with a 20 mm. cover slip will furnish a satisfactory preparation for the determination of this character if examined at once. The special staining methods used for the demonstration of flagella may also be employed. The so-called "Giant Whips" are ordinarily seen in cultures of the motile anaerobes. The presence or absence of spores and their position are also noted in the direct drop and stained preparations.

The type of growth in broth should be recorded at frequent intervals. The examination of surface colonies has already been referred to. If more than one type of surface colony is observed, it usually indicates the presence of two or more species. Frequently, however, unlike colonies ultimately prove to belong to the same species. Atypical colony form has been frequently reported but researches concerning the significance of these observations have until recently been held in abeyance. It is quite probable that investigations in this field will in the near future demonstrate that the phenomenon of microbial dissociation as found among the aerobes occurs in the anaerobic group and with like significance.

The determination of the capacity to decompose proteins and carbohydrates is quite readily detected. The proteolytic characters are judged by the extent to which coagulated serum and gelatin are liquefied. Gelatin tubes must be placed in the ice box following incubation of the culture in order to detect the extent of liquefaction. *B. sporogenes* and *B. histolyticus* are examples of the spore-forming proteolytic anaerobes which initiate protein decomposition. The degradation products include indole, skatole, phenols, ammonia, hydrogen sulphide, etc., which in combination give a profound odor to these cultures. The proteolytic strains impart a black color to the cooked meat medium, and cause digestion of the tissue.

Saccharolytic power is recognized by a definite capacity for producing acid, or acid and gas in sugar containing media. The carbohydrate to be investigated is usually prepared in a 10 to 20 per cent aqueous solution and sterilized. It is then added with aseptic technique to tubes of sterile "sugar free" beef infusion broth. It is customary to employ the sugar in a concentration of 1 per cent. After heating and cooling the medium in the usual manner previous to inoculation, the germs are introduced and incubation is carried out until a rich growth is obtained. To detect acid production a few drops of the culture are removed to a test plate and an indicator, such as phenol red, is added. It has not been found satisfactory to incorporate the indicator in the culture medium. It must also be remembered that some anaerobes produce gas from sugar-free media.

Agglutinins, precipitins, and complement-fixing antibodies can be elicited with many species of anaerobes in the blood of experimental animals, particularly rabbits, by the intravenous injection of washed bacilli. The germs are cultured in dextrose broth for about forty-eight hours, collected by centrifugation, washed three times with sterile saline, suspended in saline, killed by heat, and then injected. Each animal receives from five to ten doses at intervals of from three to five days. The saline suspension of organisms may be used equally well for testing such serums. The most contradictory results are obtained as to antibody response. Ordinarily, however, satisfactory results are obtained with cultures of *B. tetani* and *B. botulinus*.

Toxin production is usually carried out by the inoculation and incubation of dextrose broth. The time of appearance of the toxin varies with the strain and the species. Thus, with *B. welchii* the most potent preparations are obtained after eighteen to twenty-four hours at 37° C. On the other hand, it

is necessary to incubate cultures of *B. botulinus* for at least ten days before obtaining satisfactory toxic material

Antitoxins active against the respective toxins may be produced by inoculating animals with increasing doses of the corresponding toxin. The larger animals such as goats and horses, are to be preferred for this purpose as rabbits and guinea pigs are most difficult to immunize actively. It is customary therefore to obtain antitoxic serums from the commercial houses. These serums are kept on hand so as to be available for neutralization tests when injecting animals. A guinea pig is passively immunized by injection with antitoxic sera, or 1 c.c. of the culture in question is mixed with an equal volume of antiserum and left in contact for one hour at room temperature before intramuscular injection in guinea pigs.

For the determination of pathogenicity, a guinea pig is usually injected intramuscularly with 1 to 2 c.c. of a forty-eight-hour culture.

GENERAL CONSIDERATIONS

The above technique has been used over a long period of time and has been found to be simple, convenient, and satisfactory for the isolation and identification of the various pathogenic and nonpathogenic anaerobic organisms. The individual worker will quite naturally select procedures for his own particular requirements. He will find the methods capable of modification and adaptation in a variety of directions.

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TECHNIC FOR THE ISOLATION OF STREPTOCOCCI*

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WITHIN the past decade, streptococci have assumed increasing recognition as primary or secondary pathogens in a great variety of diseased conditions, and the problem of their prompt isolation and identification has become one of growing importance to the clinician. The purpose of this article is to discuss the bacteriologic procedures essential to the successful cultivation of these organisms. Many of the details described may seem elementary, but because of their importance it seems advisable to include them.

CLASSIFICATION

Since the days of Pasteur, considerable time and effort have been spent in attempting to classify the various forms of streptococci. Much of this work, while of interest to the bacteriologist, is of little practical importance. This applies especially to the defining of the fixed types of streptococci according to their power to ferment carbohydrates. Investigation has shown that there is little correlation between pathogenicity and fermentative activity. The classification of James Howard Brown is both practical and simple, and is based on the growth of deep colonies of streptococci on blood agar plates. His three main groups with their approximate synonyms are as follows:

1 Alpha type** colonies surrounded by green zones with partial hemolysis of the blood corpuscles—*Streptococcus viridans*

2 Beta type colonies surrounded by definite, clear, colorless zones of hemolysis—*Streptococcus hemolyticus*

3 Gamma type colonies producing no change in the medium—indifferent streptococci

CULTURE MEDIUMS

Many special and complicated mediums including the use of sugars and various animal tissues have been advocated for the growth of streptococci. For practical purposes, beef-heart infusion broth and beef-heart infusion agar are the most satisfactory.

Preparation of Broth—Fresh beef heart, free from fat, is passed through a meat grinder and weighed. To every 500 grams of meat one liter of tap water is added. It is then boiled, with frequent stirring, for fifteen minutes and is placed in the ice box over night. The following morning it is heated to 20° C, and filtered through a funnel bag. One per cent bacto peptone and 0.5 per cent sodium chloride are added. The mixture is then heated for a few minutes over a low flame, with constant stirring, until the peptone is dissolved. The hydrogen ion concentration is adjusted to a P_H of 7.9 with 2N sodium hydroxide†. The solution is placed in the Arnold sterilizer at 100° C for one hour and then passed through a filter paper. The P_H is again taken and is usually found to have fallen to approximately

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**Brown also describes an alpha prime type of streptococcus which is intermediate between the alpha and beta types in character.

†The authors have found the Hellige comparator satisfactory for P_H determinations.

76 If it has fallen much below this point, a further adjustment is necessary, followed by another hour in the Arnold sterilizer. The medium is placed in tubes and bottles and sterilized in the Arnold sterilizer for twenty minutes at 100° C, on three successive days. The broth is then incubated for twenty-four hours to test for sterility. The final P_H should be between 7.4 and 7.6. For purposes other than blood cultures, the medium is enriched with 1 per cent defibrinated blood.

Preparation of Agar—The agar medium is prepared in the same way as the beef heart infusion broth, with the exception that 1.5 per cent of bacto agar is added with the peptone and sodium chloride. One per cent defibrinated blood may also be added, for enrichment, prior to use.

Huntton's "hormone" broth and agar and Bailey's modification of them provide mediums rich in vitamins and are particularly suitable for the growth of streptococci. However, they have the disadvantage of being slightly cloudy and are a little more difficult to prepare.

There are a few general principles that are important in the successful cultivation of streptococci. They grow best in a slightly alkaline medium (P_H 7.4 to 7.6). The optimum temperature for growth is about 37° C. Growth is not always obtained in forty-eight hours but may occur after ten days or even longer. The tendency to discard cultures too early accounts for many negative results. Practically all streptococci grow well aerobically, but a number of them have been found which grow more luxuriantly under anaerobic or partially anaerobic conditions. The more general use of supplementary anaerobic technic is recommended.

MICROSCOPIC EXAMINATION

The microscopic study of stained smears is, of course, an essential part of every bacteriologic examination. For this the Gram method is the most useful for general purposes. The smear should be thin even and fixed by passage several times through a flame. The actual technic of staining is adequately described in standard textbooks. In inexperienced hands the results are apt to be misleading, due to insufficient decolorization, to improperly prepared, or old stains, or to uneven distribution of the stain on the fixed film. Control smears should always be made with gram-negative and gram-positive organisms and placed beside the film to be examined. Stock slides prepared with smears from actively growing cultures of *Staphylococcus aureus* and *Bacillus coli* are frequently used for this purpose. These tend however to deteriorate and lose their staining characteristics within a few weeks. A satisfactory control may readily be obtained from the debris between the teeth. This material almost always contains gram-negative and gram-positive organisms. No control method is entirely free from error as substances may be present in the original specimen that interfere with the staining process. The methylene blue stain is valuable when the study of the morphology of organisms is desired.

INOCULATING MEDIA

The best results with the use of liquid media are obtained when the amount of inoculating material compared with the amount of medium does not exceed the ratio of 1 to 10. In culturing materials such as pus and serous fluids it is advisable to seed several tubes of blood broth using amounts of the

inoculum varying from a few drops to 1 cc. Cultures containing the smaller amounts will occasionally yield positive results while those more heavily inoculated remain sterile, and vice versa. Liquid media are unsuitable for contaminated specimens or those suspected of containing more than one type of organism.

The technic for streaking blood agar plates depends on the nature of the specimen. Throat swabs usually contain a variety of organisms, and for successful culturing a thin streak is necessary. To accomplish this the swab is permitted to touch but a small area of the medium and is then discarded. A fresh swab is passed once through the inoculated area and streaked back and forth across the plate or in the form of a fan. In culturing material in which only streptococci are present the streaking may be done with a platinum loop. After passing through the inoculated area, the loop is pushed through the blood agar and a horizontal cut made. It is then withdrawn and streaked over the surface. This method permits the study of deep and surface colonies on the same plate. When the specimen is suspected of containing few or no microorganisms, the plate is streaked in the form of a fan, the swab or platinum loop being passed through the inoculated area at each stroke.

THROAT

The usual method of taking cultures from the throat is carried out with the aid of a sterile cotton swab at one end of a wooden or wire applicator. When localized inflammation exists, care should be taken to swab only the inflamed area. It is unnecessary to squeeze exudate from tonsillar crypts or to puncture them with a hollow needle, as it has been found that such procedures give substantially the same results as ordinary swabbing. The infected material is streaked on plates as previously outlined.

It should be remembered that in healthy persons green-producing streptococci may be recovered from the throat in 90 per cent of cases and hemolytic streptococci in from 10 to 20 per cent. In acute infections of the throat hemolytic streptococci are almost always present.

TONSILS

Excised tonsils which are to be studied bacteriologically should be sent directly to the laboratory without being opened by the surgeon. Each tonsil should be cultured separately. In our experience, the following technic has yielded satisfactory results. The tonsil is immersed in 95 per cent alcohol for one minute, removed with sterile forceps, shaken once to remove excess of alcohol, and placed in a sterile Petri dish. It is then cut open with sterile instruments and a piece of the central portion removed and streaked on blood agar. Another piece is incubated in blood broth. If the technic is carried out carefully, the mouth organisms are avoided, and pure cultures of streptococci are frequently obtained.

TEETH

Dental infections are usually streptococcal in origin. Before extraction, the gums should be painted with iodine and 95 per cent alcohol. In removing

the tooth care should be taken to avoid contamination from other mouth structures. The apex is immediately rubbed over the surface of a blood agar plate. The tooth is then dipped in 95 per cent alcohol for one minute and cracked open in a sterile towel with bone forceps. The interior of the root canal granuloma and abscess if present, are cultured on blood agar plates and in blood broth tubes. Pieces of tooth may also be cultured in blood broth. By this method streptococci are almost always found in diseased teeth and generally in pure culture.

SPUTUM

To examine sputum for streptococci, thin smears are prepared, stained with Gram's stain and methylene blue and examined under the microscope. Mucoid material may be streaked directly on blood agar plates. Mucopurulent masses after washing in sterile water should be emulsified in nutrient broth or saline before culturing on blood agar.

PUS

Pus may be collected with a platinum loop, sterile swab, bacteriologic pipette, or a syringe, and a needle. Smears are stained with methylene blue or Gram's stain. Cultures are made on blood agar plates and if the material is not contaminated in blood broth also. The method, previously described of inoculating several tubes with varying amounts of pus is particularly useful.

SINUSES

Sinus material submitted to the laboratory for culture is either in the form of sinus washings or of pus which the surgeon has collected on a swab after puncturing the sinus. It is assumed that the operator has attempted to sterilize the nose as thoroughly as possible before puncturing the sinus. Cultures are made on thinly streaked blood agar plates and in blood agar pour plates.

JOINT PLEURAL, PERICARDIAL, AND PERITONEAL FLUIDS

The process of obtaining joint fluid by aspiration subjects the patient to possible danger through the introduction of pathogenic microorganisms. To insure a sterile technic extraordinary precautions must be observed. Any necessary palpation for the location of landmarks should be carried out before sterilizing the skin. When the aspiration is performed at the bedside the use of sterile gloves is not advised as they tend to give the operator a false sense of security. A sharp needle of 18 or 20 gauge is selected. Sterilization of syringe and needle in a dry sterilizer or autoclave is preferable to boiling. The skin is painted with two coats of iodine and one of alcohol the iodine being permitted to dry each time before the application of the next coat. Knee aspirations are usually carried out on the medial aspect of the joint immediately below the patella. Other joints are usually aspirated at the point of greatest fluctuation. When joints contain a very small amount of fluid it is permissible to wash them out with a little sterile physiologic saline which enables the operator to obtain a specimen otherwise impossible.

With sensitive or neurotic patients it may be advisable to use a local anesthetic. A hypodermic syringe and 2 per cent novocaine from an ampule are used for this purpose. After a small bleb is made in the skin, the novocaine is slowly injected while the needle is being inserted to the joint capsule. Five to ten minutes should then be permitted to elapse in order to give the anesthetic time to take effect before proceeding with the sterilization of the skin and aspiration. Novocaine injected in this way does not interfere with the culture.

Smears are made and stained in the usual manner with methylene blue and Gram's stain. In addition it is sometimes advisable to use Wright's, or some supravital technique, for study of the cell content. Cultures are made in blood agar and blood broth as previously described.

The bacteriologic examinations of pleural, pericardial, and peritoneal fluids are carried out in the same way as for joints.

URINE

In culturing the urine, it is necessary to use a catheterized specimen, and considerable attention should be paid to the sterilization of the urethral orifice. Cultures are made on blood agar plates and in blood broth tubes. It is always advisable to inoculate several tubes of blood broth with varying amounts of urine.

GENITAL TRACT

Bacteriologic examination of the female genital tract, other than the uterus, is usually made by studying smears stained with Gram's stain and methylene blue, and from cultures on blood agar plates. Examinations of the uterine cavity are complicated by the possible danger to the patient through the introduction of pathogenic microorganisms. The technique of Harris and Brown is the method of choice for cases of suspected puerperal sepsis. According to this procedure, the external genitalia are painted with 2 per cent alcohol-acetone solution of mercurochrome. The cervix is then exposed with a sterile bivalve speculum and wiped dry with sterile gauze sponges. A glass lochial tube of the type described by Little is inserted in the uterine cavity and carried to the fundus. When the tube is filled, it is removed and immediately taken to the laboratory. The tube is broken with the aid of a file and the middle of the column of lochia is examined by smears and culture. Blood agar plates and slants sealed under reduced oxygen tension, human serum bouillon and cooked meat medium sealed with vaseline are used. The last medium gives the best results. It must be borne in mind that streptococci are occasionally found in the vaginal secretions of normal females.

STOOLS

The culturing of stools is complicated by the presence of various kinds of bacteria, in large numbers. Many methods for growing streptococci have been advocated by investigators. The principles generally employed are either the dilution of the stool or the use of various reagents to inhibit the growth of unimportant organisms. Probably the most common technique calls for the dilu-

tion of the stool in physiologic saline before culturing on blood agar pour plates. In our experience, the following method has been found to be most satisfactory when only the streptococcal content of the stool is desired. A representative specimen of stool the size of a pea is emulsified in a test tube containing 10 c c of a 1 per cent solution of sodium carbonate and allowed to incubate at room temperature for twenty-four hours. Subcultures are then made on blood agar pour plates, using varying amounts of the inoculum for each plate. The cultures are then incubated for twenty-four to forty-eight hours at 37° C. This method eliminates all the bacteria usually found in stools except the streptococci.

It must be remembered that 90 per cent or more of stool specimens from normal persons contain green-producing streptococci while 10 to 15 per cent of them contain hemolytic streptococci.

The streptococci found in the stools fall roughly into two groups, the so-called true streptococci and the enterococci. The latter may be distinguished by the following characteristics which are not found in the first group:

- 1 They ferment mannitol (Andrewes and Horder)
- 2 They grow well in bile-glucose-peptone broth (Weissenbach)
- 3 They are heat resistant (Dible)
- 4 They are able to split esculin (Harrison and van der Leek)

SKIN

Two methods are particularly efficient for the recovery of streptococci from the skin of erysipelas patients. (1) Fehleisen, after sterilizing the surface of the periphery of the lesion, excises a portion of the skin and cultures it in blood broth. By this method, a hemolytic streptococcus is readily recovered. (2) Birkhaug has successfully eliminated the necessity of an operative procedure by injecting 0.5 c c of sterile saline into the skin at the edge of the lesion, later, aspirating and culturing the fluid.

In culturing skin conditions where contaminations are present the Sabouraud bouillon pipette method and Haxthausen's crystal violet method are satisfactory.

Sabouraud cultures the serum exudate or an emulsion of scales from the lesion, using aseptic fluid with bouillon in Pasteur pipettes. This method permits aerobic conditions in the wider part of the pipette and semi-aerobic conditions in the capillary stem. The latter is a more suitable environment for streptococcal growth while it is unfavorable for the multiplication of staphylococci. After a period of twenty-four hours' incubation, the fluid in the stem of the pipette is examined and subcultured. In this way a pure culture of streptococci may be obtained from a mixed infection.

Haxthausen's technique allows for a quantitative estimate of the number of streptococci present while at the same time it eliminates contaminating organisms. The medium he uses is a 10 per cent blood agar in which crystal violet in a concentration of 1:100,000 is added. This investigator states that in nine out of ten cases any streptococcus will grow in this medium while staphylococci are entirely, or almost entirely, inhibited both when the culture is made from fluid material and when scales are sprinkled over the agar. A liquid bouillon medium may also be used with the same concentration of the dye.

TISSUES

The usual methods of staining bacteria in tissue sections are unsatisfactory in that frequently gram-negative organisms are not decolorized and that the bacteria are not clearly differentiated from tissue elements. Brown and Bienn have recently described a method that largely eliminates these difficulties, decolorization being accomplished with a solution of picric acid and acetone. Readers are referred to their original article for details.

Cultures are made by placing pieces of the tissue in blood broth and macerating them with a glass rod. Tough material may be ground in a mortar with sterile sand, preferably moistened with a small amount of bouillon, and then cultured in blood broth and blood agar. The grinding process, even under suitable conditions, exposes the tissue to possible contaminations and should be used infrequently.

MILK

In epidemics of septic sore throat the clinical laboratory is sometimes called upon to examine samples of milk. Blood agar pour plates are generally used for this purpose. Hardenbergh's technique is satisfactory. Samples from individual cows are generally cultured in dilutions of 1:100, composite samples in dilutions of 1:20. For each plate, 1 c.c. of the diluted milk is used and the culture is incubated from eighteen to forty-eight hours. Organisms suspected of being *Streptococcus epidemicus* are tested with a medium containing sodium hippurate. *Streptococcus epidemicus* does not split nor hydrolyze the sodium hippurate, while the other streptococci do. In addition, the *Streptococcus epidemicus* has a capsule.

The estimation of the final hydrogen ion concentration of the medium is valuable in differentiating the human and bovine types of *Streptococcus hemolyticus*. If grown in 1 per cent dextrose broth the streptococcus of human origin will give a final P_H of 5 to 5.3 and that of bovine origin 4.3 to 4.5 (Avery and Cullen).

BLOOD AND SPINAL FLUID

Blood and spinal fluid cultures are described in a separate article.

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PREPARATION OF VACCINES*

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SINCE the introduction of bacterial vaccines as therapeutic agents, their use has undergone the vicissitudes which not infrequently have attended the introduction of various other preparations in medicine. Frequently exploited by the commercial laboratory, hailed by the therapeutic faddist as a "cure all," used blindly in the treatment of almost every conceivable ailment,¹ and often as a last resort in some obscure condition where other therapeutic means had failed, reaction naturally followed, condemnation often replaced praise, and their general usage diminished. Nevertheless, excellent therapeutic results were occasionally observed in the treatment of certain conditions which encouraged serious study of the problem by some investigators. The continued investigations of scientifically trained bacteriologists and immunologists in conjunction with discriminating clinicians have advanced the preparation and application of bacterial vaccines to a rational basis, and established their place in therapeutics among other well recognized biologic agents. Recent discoveries in the fields of bacteriology and immunology have shed light upon some of the past failures, and have tended to clear the problem. As knowledge has been gained concerning bacterial variations, and their relationship to antigenic properties, advances have been made in the preparation of more efficient products. Methods of cul-

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ture have improved, and the selection of the proper type organisms to enter the vaccine has become possible

Experimental evidence indicates that the antigenic properties of bacteria are dependent upon certain chemical components of the cell, even associated with certain differentiated portions of the cell, the chemical nature of certain of these cellular products is known,² and the problem of specificity has been elucidated in certain instances

Obviously, the desired objective in the preparation of an autogenous vaccine is to culture the organism under conditions which produce the least alteration in the finer chemical structure of the harmful parasite as it existed in the host, arresting or destroying its vitality with the least alteration of that chemical structure, and retaining the intrinsic composition of the antigenic components of the cell in that state in the prepared vaccine

In the following article on the properties of autogenous vaccines it will be noted that, insofar as practical, the above mentioned principles have been applied. The methods employed have been the outgrowth of years of experience in this field and have given satisfactory results. As knowledge advances, improvement in methods must follow, procedures now practiced may be found inadequate when viewed in the light of future investigations. Emphasis must be given to the importance of the strict supervision of the entire procedure by a well trained bacteriologist and immunologist. The average laboratory technician does not possess the training, nor the discrimination essential for the best results

In recent years the use of autogenous vaccines as a means of treatment has been extended to include certain allergic conditions, possibly due to focal infections. Therefore in outlining a method of procedure, it is necessary to consider in detail foci of the respiratory, the gastrointestinal and the genitourinary systems. A complete bacteriologic survey of the patient should be undertaken in most instances to secure the offending organisms

Collection of Specimens—Materials are collected from the nasal passage and accessory sinuses, either by aspiration under aseptic precautions, or by means of a sterile cotton swab passed along the floor of the canal after the patient has vigorously blown the nose on sterile wipes. A sterile nasal speculum should be used to dilate the nostrils, when swab specimens are taken. Secretions, etc., of the nasal pharyngeal area are collected by means of the West tube. In taking specimens for culture from the faucial tonsils, pains must be taken to express pus from possible pockets and deeply penetrate the crypts to secure caseous materials, etc. The ordinary cotton swab (sterile) usually answers all purposes. Occasionally the lingual tonsils are involved, therefore they should be examined in the routine procedure. Early morning specimens of sputum or that following paroxysmal coughing attacks should be collected in a sterile jar and kept on ice until cultured

Röntgenograms of the teeth should be made and when evidence exists of apical infections these teeth may be extracted under aseptic conditions and cultures made. If possible material should be taken from the socket and cultured on suitable media immediately at the time of extraction. Cultures should be made from gum lesions where pyorrhea is evident

In some instances gall bladder infections are suspected. Bile specimens may be secured for culture by passing the duodenal tube and allowing it to remain until sufficient fluid is collected for cultural purposes. Aseptic precautions must be taken throughout to exclude, insofar as possible, extraneous contamination.

Fecal material for culture may be obtained by means of high colonic irrigation with sterilized water, or from the last movements following the administration of castor oil, or one of the saline cathartics.

Urine specimens may be secured by catheterization, or following normal passage, after having cleansed the external orifice with soap and water, and then rinsing thoroughly with sterile saline solution.

Not infrequently the vagina and the cervix are sites of infection, and should be routinely cultured in those suffering from chronic arthritis. The patient is placed in the proper position for examination, a sterile vaginal speculum is inserted and by means of long cotton swabs, two sets of cultures are made, one from material taken from the vaginal fornix, the other from the os uteri.

In the male, the prostate may be involved. In such conditions, the external parts are thoroughly cleansed with soap and sterile water, then the gland is massaged, and the fluid collected on sterile swabs as it passes, then cultured at once, if possible.

Other possible foci of infection may be found and cultured. But those just mentioned constitute the group which must be considered in a general bacteriologic survey of the patient suffering in particular from an allergic symptom complex.

Culturing of Materials—Before culturing materials from any source, if a sufficient amount of the specimen permits, a preparation stained by the Gram method is studied microscopically, and the various types of organisms present are noted, as well as their quantitative relationship to each other. As a routine procedure, materials from the respiratory tract are cultured directly in dextrose broth on blood agar, and on hormone agar slant. Human blood is preferable in the preparation of the agar slant, and in the Avery medium. When possible, sputum is washed through three changes of sterile physiologic salt solution, then the washed masses are placed in the culture media. Also the washed sputum, pus, feces, etc., are emulsified in broth, then immediately plated with blood agar by the poured plate dilution method. Feces are cultured on plain and dextrose broth, and in 1 per cent sterile sodium carbonate solution, this solution in amounts of about 10 c.c. is placed in the ordinary test tube and sterilized in the usual way. Each tube of medium receives a quantity of material equivalent in size to a navy bean. Vaginal and cervical materials, and prostatic fluid, are cultured directly at the time of taking in dextrose brain broth (Rosenow). The medium is first placed in a cup of water, boiled for ten minutes, then cooled to 37° C. or less, before the material is added for culture.

After eighteen to twenty-four hours' incubation at 36 to 37° C., the growth in the various culture tubes is studied microscopically from prepared films stained by the Gram method, and the morphologic types are noted in each instance. Then the cultures are plated by the poured plate, dilution method with blood agar, for isolation of the species present. After proper incubation of the plate cultures, the colonies are studied, then general characteristics noted,

including their hemolyzing properties "rough" or "smooth" appearance etc and then fishings are made to suitable media Those resembling the streptococcus and the *M. catarrhalis* type colonies are cultured on dextrose broth, the staphylococcus type on plain agar slants, and the coliform colonies on both dextrose lactose agar (Russell) and saccharose mannitol agar (Kendall) for a preliminary differential test Materials such as sputum, pus etc which were emulsified and directly plated, may yield organisms in pure culture by the second generation to be converted into a vaccine while those first cultured on broth or agar media before plating usually undergo three or more generations before they can be prepared as vaccines

On a priori grounds it would appear important to immediately convert the organisms isolated from the patient into a vaccine for his treatment on the supposition that the relation of organism to the host was that of a highly parasitic character which would tend to be lost if the organism was grown a number of generations upon artificial medium, a saprophytic existence It would appear that much of the finer specificity might be lost as the organism readjusted itself to a changed environment For some years this principle in the preparation of autogenous vaccines has been practiced³ and probably with beneficial results In limiting the number of generations of the organism as derived from the patient, the tendency to variation or mutation would be much lessened, a thing of theoretical importance and probably of practical significance Also in making fishings of colonies from plates, only the smooth type is selected on the basis of their specific antigenic properties Again, in some instances the question of oxygen requirement is important, and requires special handling of the organism

Cultures for Vaccines—The antigenic substances of bacteria are assumed to be associated with the nucleoprotein of the cell and the capsular and the flagellar structures when they are present In culturing bacteria for vaccines a selection of media which favor the development of the organism to its fullest extent is desirable However certain cultural media ordinarily used for such purposes might introduce substances of a harmful nature and therefore should not be used As a routine the staphylococcus and the coliform groups are grown upon plain agar slants although the latter group may be grown upon the double sugar media of Russell and of Kendall, the streptococcus the *M. catarrhalis* and the diphtheroid groups are grown upon dextrose broth flasks containing approximately 75 c c of fluid *B. influenzae* is grown upon 5 per cent human blood agar

As mentioned above the first or second generation of the organism should be used for seeding purposes and as early as possible in its development Incubation at 36° C is found to produce excellent growths usually in eighteen to twenty-four hours in properly prepared media whose reactions are correctly adjusted Often it is advantageous to place the freshly seeded media in a water-bath at 36 to 37° C to hasten the growth Young vigorously growing cultures eighteen to twenty hours old should be used for the preparation of the vaccine thus holding to a minimum autolytic disintegration the deleterious effects of acid production etc The use of old cultures for seeding purposes or for the preparation of vaccines should not be permitted

Preparation of the Bacterial Vaccine—A modification⁴ of the volumetric method of Hopkins⁵ has been used in our laboratory for a number of years with highly satisfactory results. This method possesses the advantage of simplicity of technique, rapidity of results, relative uniformity of concentration, etc., which commends its use from the practical standpoint. The only special piece of apparatus required is the Hopkins centrifuge tube,⁵ which may be procured from any of the leading scientific supply houses. However, it is necessary that these tubes meet the specifications laid down in the original article by Hopkins. The other apparatus used may be found or made in any well-equipped laboratory. No attempt will be made to enter into details in this article, as they have been published elsewhere,⁶ but only a general outline of the procedure will be given. At the outset it must be stated that only sterile glassware, solutions, etc., may be used throughout, and strictly aseptic precautions exercised in each step. If the organisms are grown on agar slants, they should be emulsified in a small portion of physiologic salt solution. The bacterial suspension is removed by means of a Pasteur pipette, then strained through cotton in a suitable filter funnel, and collected in a Hopkins centrifuge tube. The filter funnels are prepared by drawing out test tubes, or using a Hopkins tube whose tip has broken off, and placing a small tuft of absorbent cotton in the broken end. Both the filter funnel and the Hopkins tubes are placed in larger test tubes, which are sealed with cotton, and sterilized by dry heat before using. If the organisms have been grown in broth flasks, the sediment is removed directly by means of a sterile pipette, and collected in the Hopkins tube. The tubes are sealed with gauze-wrapped cotton plugs, which are securely fastened in position, then placed in the centrifuge. The organisms are thoroughly packed in the collecting portion of the centrifuge tube after one-half hour in a machine carrying a head of 18 cm diameter, driven at 2800 to 3000 revolutions per minute. After sedimentation is completed, the supernatant fluid and any excess of bacterial sediment are removed by means of a Pasteur pipette and rejected. Sufficient physiologic salt solution is added to yield a 1 per cent suspension of the bacterial residue, and a homogeneous suspension is made by thoroughly mixing with the pipette. The bacterial suspension is transferred to a sterile test tube containing a few small beads, which is thoroughly heated in a Bunsen flame from the mouth downward about one-half its length, then is sealed with a sterile cotton plug.

Killing of the Organisms—Much has been written concerning the best methods of treating the organism before its introduction into the body of the individual to be immunized, whether the organisms should be simply attenuated, or should be killed.⁷ Owing to the attending risks of infecting the recipient, attenuated cultures have fallen into disuse. At the present time, practically all investigators are agreed upon using organisms whose vegetative powers have been destroyed, even if the living organism theoretically possesses greater antigenic possibilities. As to the best method of arresting or destroying the vegetative powers of an organism and still retaining its highest antigenic value, much disagreement exists among different workers. Whether this should be induced by physical means, such as the production of autolysates⁸ or by the use of heat, or,

on the other hand, by chemical means,⁹ such as by germicides still remains to be decided. The chemical group embraces a rather large number of substances each appearing to have its special advocates.¹⁰ In particular, many hold that chemical means of killing organisms is superior to the use of heat¹¹ but the published studies are not fully conclusive since the claims are frequently based upon animal experimentation and studies of certain immune bodies or serologic reactions, which have a questionable relationship to the true immunologic processes occurring in the patient undergoing protective, or therapeutic immunization. The whole subject requires restudy, taking in consideration certain biologic reactive processes more closely associated with pathologic conditions such studies are now under way. Therefore, until more conclusive evidence than that which has been presented up to this time is forthcoming the heat-killing method will be retained in preparing the vaccine. But as a basic condition, only the minimal amount of heat is used which just kills the organism after one hour exposure. In general 58° C suffices for the true streptococcus group and some of the gram-negative cocci, but for the coliform group and the staphylococcus group, a higher temperature is necessary, and even a temperature reaching 64 to 65° C may be required to insure the killing of the enterococcus (*M. ovalis*). The sterilized test tube containing the physiologic salt solution suspension of the organism is submerged to a depth of about one inch from the mouth in a water-bath, then heated for one hour at the required temperature. A separate vaccine is made from each of the organisms isolated from the various foci cultured in the patient.

Control of Vaccine—After heating the suspension of the organisms as stated, the tube is removed from the bath and shaken thoroughly to ensure a uniform mixture, then two or three drops are planted into each of two culture tubes one containing about 20 cc dextrose broth the other an agar slant. These tubes are incubated at 36 to 37° C for seventy-two hours to determine the sterility of the vaccine. After making cultures for the sterility tests, the remainder of the vaccine is transferred to a sterile, 15 cc amber glass bottle, containing a few small glass beads, and sufficient 10 per cent emulsion of tricoresol in distilled water is added to give a final dilution of 0.25 per cent. The bottle is sealed with a sterile rubber stopper properly labeled, then well shaken and stored in the refrigerator. When the sterility of the preparation has been proved the vaccine is ready for use. The vaccine in the storage bottle is known as the "stock vaccine" and contains practically 1 per cent of the killed bacterial substance.

Standardization of Vaccine—Hopkins estimated the average number of organisms present in 10 cc of 1 per cent suspensions of various bacterial species prepared by his technique and established those numbers as "standards." Dilutions of the "stock vaccines" were made for therapeutic purposes, and the bacterial concentrations were expressed in numerical values.

In a study of the application of autogenous vaccines to the diagnosis and treatment of asthma¹² it was found that the numerical method of standardization as employed by Hopkins was not as practical as could be desired. Therefore a modification⁴ was introduced which proved more satisfactory. The standard adopted was based upon the volume content of moist bacterial substance col-

lected in the Hopkins tube under standardized procedure. The *vaccine unit** may be defined as consisting of that volume of bacterial substance (killed) represented by 0.01 c.c. of a 1 per cent suspension, prepared under certain required conditions. This quantity (one unit) of vaccine has been found the most favorable amount to produce a positive skin reaction¹³ in the hypersensitive individual when given intradermally, the absence of a reaction eliminates a patient as nonsensitive to the organism, insofar as the direct skin test indicates. The particular advantages of the volumetric vaccine unit are greater uniformity in dosage, constant valuation of vaccines prepared from the same species, and comparable valuations of vaccines prepared from different bacterial species.

Testing Vaccines—Vaccines for the intradermal test of the patient to determine his reaction to the various organisms (killed) isolated from material taken in the bacteriologic survey, are dispensed from the 1 per cent sterile stock preparation. They are supplied in 1 c.c. ampoules, either as the original 1 per cent suspension, or a dilution¹⁴ of one part of stock vaccine in five parts (1:5) of the standard diluent, consisting of sterile physiologic salt solution containing 0.25 per cent tricesol. Usually 0.5 c.c. of either suspension is placed in the sterile ampoule which is sealed with sterile rubber stopper, then properly labeled, and kept in the ice box until used. The dose used in the skin test consists of 0.01 c.c. of the 1 per cent concentrate, or 0.05 c.c. of the diluted stock vaccine, the larger volume permits more accurate measurement.

Therapeutic Vaccines—The preparation of the treatment vaccine is dependent upon the results of the skin reaction following the intradermal injection of the test vaccines. One or more bacterial species may be indicated as etiologic factors in the patient's condition, so the respective vaccines are selected accordingly for therapeutic use. They may be dispensed separately, or may be combined in different proportions as desired. Usually the concentration of the autogenous vaccine, primarily supplied, contains 10 units of the bacterial substance per cubic centimeter, but it may become necessary to alter this concentration after the first injections, depending upon the patient's general reaction to the treatment. The dilutions of the treatment vaccines are made from the "stock vaccines" by accurately measuring out the required amount of the uniform suspensions after thoroughly shaking, with a sterile 1 c.c. pipette marked with 0.01 c.c. graduations. The given amounts of the respective vaccines are placed in a sterile 10 c.c. vial, containing a few small glass beads, then that amount of standard diluent (see above) is added to bring to required volume, sealed with special sterile rubber stopper, then properly labeled. The vaccine is kept constantly in the ice box except when in actual use.

In presenting this discussion on the preparation of vaccines it is with the knowledge that certain procedures may be subject to just criticism, as much difference of opinion exists among different investigators as to the relative merits

*Occasionally certain individuals are found to be extremely hypersensitive to certain bacterial vaccines and grave reactions follow the initial dose. In such cases greatly reduced doses must be administered to them during the course of their treatment. As more or less difficulty may be experienced in dealing with fractional unit dosage it has been suggested by Dr. William S. Thomas that a *milliunit* of measurement might be recognized and thereby the dosage be expressed in whole numbers in that particular group of cases. By the *milliunit*, it is understood that the original whole unit as defined above is simply subdivided into one thousand parts and each of these parts designated as a *milliunit*. The doses of vaccine administered to the patient during the course of treatment would be recorded on his chart in terms of milliunits rather than in fractions of the basic unit.—Personal communication.

of one or another method of arriving at a given result. Ultimate results have appeared to justify the methods outlined in the foregoing presentation. Revision may become necessary if further investigations definitely decide certain doubtful questions, and show their superiority to procedures now in vogue.

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VACCINES IN CLINICAL MEDICINE

By ROBERT A. KEILTY, M.D., WASHINGTON, D. C.

AT FIRST thought it would seem that a well rounded paper on the subject of vaccines would be both timely and easy to present. The difficulties and uncertainties of vaccines and vaccine therapy and the wide divergence of experimental and clinical opinion accounts for an imbalance in the position of the whole subject at the moment. We may rule out of this discussion the active immunity of vaccines produced in the prevention of such diseases as typhoid fever, since this is an established accomplishment and we are no longer living in the age of Chickamauga Park as far as typhoid fever is concerned.

You either believe or you do not believe in vaccines. This attitude may be well grounded from actual first hand experience; it may be a belief fostered in ignorance, or it may be the result of a teaching which you follow. There is too much accomplished by the intelligent use of vaccines¹ to entirely disregard them as is the custom of many. At the same time the problem has not been basically nor sufficiently elucidated experimentally and the results are not constant nor consistent enough clinically to put the question on a firm foundation of fact. One is torn between the intricacies of theory and the results obtained in practice.

If you do not believe in vaccines you either have no interest in them or at most only a passing fancy. If you do believe in them you may be a hyperenthusiast. Both of these positions are untenable. I believe that the midground

between two extremes is always a safe place to work in, and this is certainly true for the vaccine problem. In other words, stay in the fair way, keep out of the rough but keep playing and do not pick up

What is a vaccine? A vaccine is a buffered solution of immunogenic or vaccinogenic bacterial substances, bacterial bodies, toxins, phages, and filtrates prepared in such a way that the greatest chance for obtaining and maintaining specific antigenic or stimulus body is preserved intact. To begin with, there are all manners of vaccines, prepared by all sorts of methods and so with great variation of antigenic substances. If possible, the properties of the antigen or vaccinogenic substance or substances, whether or not of protein nature, must be accurately determined and measured with better methods for the recognition and dosage devised.

At the present time I am assuming that the isolation of a single organism from a given lesion, as for example, the hemolytic white or yellow staphylococcus from a cutaneous abscess (boil or furuncle), in pure culture, is the etiologic stimulus for that infection, and that its combat may be accomplished by the production of antibodies produced in the body which are more or less specific against it. It is well recognized that a properly prepared vaccine, autogenous or stock, will effect prompt results in a large number of these cases. Against this there are many failures with recurrences. In these cases of failure, there is a chance for the study of the antigen which is probably quite specific or at least the conditions for specificity are quite apparent, and the effects of modifications of vaccine preparations can be nicely studied and the results interpreted.

In the case of multiple organisms in a flora, in which one or more may be the etiologic factor, the problem is more complicated but the same principles may be applied to each organism isolated as though dealing with a single organism infection. The best example for multiple types is the flora from the sputum and upper respiratory tract in pulmonary infections. Many excellent results are obtained in the use of vaccine in bacterial asthma and chronic bronchitis and bronchiolitis. In these cases I plant the material by light streaks on blood agar plates and select colony types in order of their quantitative growth. From these first transplants, salt solution preparations are made and sterilized, keeping each type separate. These usually average four, five or six. Where a number of different sources, feces, urine, prostate, cervix, gingiva, are suspected as foci, isolations are made in the same way. This may amount to twenty or more individual preparations. The organisms to be used in the final vaccine are picked out according to the intradermic, allergic reaction of each. This reaction must amount to 50 per cent or more and is read immediately, within one-half hour, and again for delayed reaction in twenty-four hours. Only those organisms showing definite cutaneous allergic reactions are considered for use. This principle of restricting the use of possible etiologic bacteria to only those which give positive allergic intracutaneous reactions may eventually be shown to rule out some organisms having a vaccinogenic substance but my own results during the past year have been improved I think because of this procedure.

A vaccine, therefore, must be started if possible from the actual specific etiologic organism. It must be prepared, utilizing bacterial bodies and soluble bacterial products in such a way that their antigenic or vaccinogenic factor is

altered in the least possible degree. No matter how desirable a living organism might be from the standpoint of its vaccinogenic value I believe it should be killed. I am still heat-minded, using the smallest amount for the least time with the addition of tricresol. The final control from the finished ampoules must be sterile using the same medium or media as in the original isolation. I realize the possible value of attenuated forms but I still fear their potential possibilities.

The dosage of a given vaccine varies with each one given. The present trend is to start with very small doses, at least 0.1 c.c. and increase in 0.1 c.c. stages keeping the dose just short of local or general reaction. I believe that general reactions (malaise, some headache, slight fever, and an increase in focal symptoms) are not only to be desired but are essential if the fullest degree of immunologic factors are to be obtained. I pay very little attention to the local reaction unless it is severe, when I delay the next dose for a day or two, but I do not consider a maximum dose until a general reaction has repeated itself at least two or three times. The local reactions are so uncertain, may be severe with the first dose or not at all with much larger doses, that they cannot be used as an intelligent guide to dosage. General reactions, on the other hand, are quite definite and appear a few hours after injection. I try to get a result comparable to that of a typical antityphoid reaction.

Bearing in mind my method of bacterial selection and preparation with my reaction to dosage, I pay no attention to bacterial counting in the original vaccine but always use heavy suspensions, so that my vaccines are always milky cloudy as compared to the light almost watery preparations of most laboratories.

Vaccines are wonderful adjuncts but are not cure-alls. They must be given with the definite purpose of raising the immunologic bodies against a given infection, if our theories on immunity have any basis, in fact, but that infection must be reduced or eliminated wherever possible by all other means available. A great many of the indifferent results and failures can be charged, not only to the nonspecificity of the vaccine used, but to the fact that vaccines alone are incapable of combating the conditions of infection.

The selection of cases for the use of vaccine therapy is also of great importance. In all types of infection, direct or focal a vaccine is indicated, and good results may be expected in a high percentage. This percentage of results is improving as the selection of cases is more carefully made as the vaccines are more intelligently prepared, as the dose is more adequately administered as the originating foci are more thoroughly eliminated and as direct chemotherapy where indicated is more specifically applied. Thus, it seems to me the vaccine situation of today is summed up.

Shall vaccines be given intracutaneously, subcutaneously, intramuscularly, or intravenously? The intracutaneous method will undoubtedly give vaccine results but the dosage must be smaller. I am not in a position to recommend it exclusively at this time. The subcutaneous method has been and is largely preferred but for the past year or more I have been using a deeper intramuscular injection. This can be done with much less annoyance to the patient, less local reaction and with as efficient vaccine results. It seems to me that eventually the intravenous route will be the method of choice but as long as bacterial bodies are used they might become embolic and might initiate thrombosis.

The giving of a dose of vaccine is an art. Good, new, sharp needles are essential, the syringe, of course, must be sterile. The proper amount of vaccine is drawn into the syringe after sterilizing the top of the bottle with alcohol, the surface of the arm or leg is thoroughly cleaned with sterile gauze, alcohol and dried. The area selected over a muscle, in the arm the triceps, is gently held with the left hand and the needle quickly plunged into it. The contents of the syringe are injected as quickly as possible, the needle withdrawn and the area gently massaged with a sterile sponge. The giving of a small dose should be accomplished in less than a second and even one cubic centimeter in two or three seconds. This rapid method is approved and appreciated by the patient. As expressed by Kolmer, I think the result of vaccine dosage is greatly enhanced in the hands of those with increasing experience.

CONCLUSIONS

The position of vaccines, theoretically, experimentally, and clinically is today one of imbalance.

There has been too much accomplished in the past to disregard them entirely and a middle ground is an acceptable attitude.

A vaccine is defined as a vaccinogenic solution having the power of producing specific antibodies *in vivo*.

Vaccines prepared from a multiple flora are selected by me exclusively on their quantitative appearance in culture and their allergic cutaneous reactions.

Vaccines must start out with the study and isolation of specific etiologic organisms.

Vaccines must be prepared in such a way that the vaccinogenic factor is as nearly unaltered as possible, but I am not in favor of using any solution where a viable organism has any chance of becoming virulent.

The present trend in dosage is to start with small amounts, 0.1 c.c. increased in 0.1 c.c. stages and given every other day over a long period. Each individual case is an entity and for this reason the best results are obtained after experience.

In the preparation of a vaccine, I pay no attention to the bacterial count but make heavy suspensions and vary the dosage according to general reactions.

The desirability of general reactions for immunity producing purposes is stressed.

Vaccines are wonderful adjuncts but not cure-alls.

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EXAMINATION FOR PATHOGENIC FUNGI*

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FUNGI are those plants of the Thallophytes which are devoid of chlorophyll and must, therefore, depend for their food upon vegetable or animal tissues. In the examination of any material for fungi there are two modes of attack: one is the direct examination of the material, and the other is the cultivation of the organism from the material. In the first case the material, either stained or unstained, may be examined with the aid of the microscope. In general wet (unstained) preparations are preferred to stained ones because the morphology of this group of organisms is so characteristic in the unstained condition. Pus cells and fibrin, in exudates, and the tissue cells in tissue sections generally obscure the field, therefore the material is best placed on a slide and a few drops of antiformin, 20 to 40 per cent sodium hydroxide, or alcohol sodium sulphide¹ is added to the material on the slide, covered with a cover slip and examined. If scrapings from the skin are to be examined the alcohol sodium sulphide will be found to be superior to the antiformin or the sodium hydroxide. The sodium sulphide will clear the specimen in a few minutes, where the time necessary to obtain a satisfactory slide with the hydroxide will require several hours in contact with the solution. In either case I have found it profitable to examine the slide the next morning, adding water, if necessary to replace the loss due to evaporation. Evaporation may be retarded by placing the slide in a Petri dish containing moist filter paper. It must be borne in mind that certain fungi are dissolved in sodium hydroxide.

In the normal and abnormal tissues of man and animals there are fibers very similar to the filaments of mycelium. The mycelium reported in sections of the spleen in cases of splenomegalia is an example of this.

NOMENCLATURE

When the mycelium is not divided into cells by partitions it is said to be continuous, nonseptate or without septa. When the mycelium is divided into cells it is said to be septate or noncontinuous.

Cells containing many nuclei are known as coenoerytes.

Hyphae are special branches which bear spores. The simplest forms of spores are bud-like outgrowths on the mycelium and are called gemmae. This is well illustrated in the yeasts. The mycelium may be cut off by partitions and the protoplasm inside gathered into a mass which has a thickened wall known as chlamydospores. Spores which are cut off from the tip of hypha are known as conidia or conidiospores and the hypha bearing them is a conidiophore. Spores in chains are said to be catenulate due to the development of one spore below another before the elder spore is cast off. Conidia composed of one cell are described as being simple while those composed of two or more cells are compound.

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Spores may be contained loose inside of swollen tips of hyphae. These spore cases are sporangia and the hyphae are sporangiophores. The Mucors are of this type. The ascus differs from the preceding in that the number of spores is generally of a definite number, eight being the most common, although they may be lesser or greater, they are of the order 1, 2, 4, 8, 16, 32, etc. The asci may be naked or covered, scattered or collected into groups. When naked the cup or disk which bears the ascus is known as an apothecium, when covered the fruit is a perithecium.

Sexual spores are formed by the fusion of sexual elements known as gametes. The Oomycetes reproduce in this manner. Arthrospores are the fragmentation of the mycelium and are also known as Oidia. *Oidium lactis* is an example. Aleurispores are attached to the mycelium and differ from conidia merely in their place of attachment. Hemispores are structures which appear to be a transition between the yeasts and the oidia, and conidia, some of the cells of the mycelium develop a swollen structure which later breaks up into a series of spores resembling arthrospores. *Aleurispores differ from conidia in that they are not set free when mature, but are only liberated when the mycelium that forms them disintegrates.* These structures are analogous to lateral chlamydospores.

DETERMINATION OF MORPHOLOGY

The mycologist identifies fungi by morphologic methods and for this method Petri dishes and slide cultures are to be preferred to those of the culture tube. The growths on the medium in the Petri dishes are first examined with the unaided eye, then with a hand lens. A lens of 4× is very good for this work. Following this the growth may then be examined with the 16 mm objective, using the microscope. Considerable information may be obtained by inverting the dish, without removing the cover, and examining with this objective. This keeps the culture free from contamination but the arial hyphae cannot be examined with the clarity that is possible with the cover removed. The 4 mm objective may be used to a limited extent, with the cover removed. By these methods the fruiting organs may be examined with considerable detail. The finer details of the structure may be seen by removing some of the growth to a slide, mounting in water, applying to cover slip and examining with the higher powers. By these methods the structure of the mycelium (whether it is septated or not), the structure of the sporophores, the position of the spores, together with their shape and number, the presence or absence of chlamydospores, and other morphologic characters may be studied. Pleomorphism of fungi due to variation in culture media and the age of the culture should always be kept in mind.

CULTIVATION OF FUNGI

The best medium for the isolation of fungi, provided the sought-for species will tolerate the acidity, is one made as follows: one per cent peptone and one and one half per cent agar in water, distribute among culture tubes in 10 c c quantities, plug with cotton and autoclave. Prepare an aqueous solution of 50 per cent dextrose and 5 per cent tartaric acid and autoclave. When required for use melt the agar and add 1 c c of the dextrose, tartaric acid mixture. This

gives a concentration of about 5 per cent dextrose and 0.5 per cent tartaric acid. The reaction is about $P_H 4$. Most fungi will grow luxuriantly on this medium, while the bacteria are almost completely retarded.

Sabouraud's medium is extensively used for the cultivation of fungi. This medium contains 1 per cent peptone, 4 per cent crude maltose and $1\frac{1}{2}$ per cent agar. Sabouraud's "proof agar" is made by using a particular brand of peptone and crude maltose. For most work any brand will suffice and dextrose may replace the maltose.

Wort and aqueous extracts of numerous vegetables are used to grow fungi as are also the vegetables themselves prepared after the manner of the potato culture used in bacteriology.

THE MORE IMPORTANT FUNGUS DISEASES

Actinomyces The majority of these infections present grains in the pus. When grains are crushed on a slide and stained with one of the aniline dyes, or by Gram's method, they will be seen to be made up of fine threads, generally gram-positive. A great many members of this genus are aerobic, but some are anaerobic, therefore in plating the cultures should be grown aerobically and anaerobically. Growth is generally slow. The organism may be demonstrated in the tissues by histologic methods using eosin and hematoxylin or other stains. The characteristic club forms are seen in the tissues (Fig. 13).

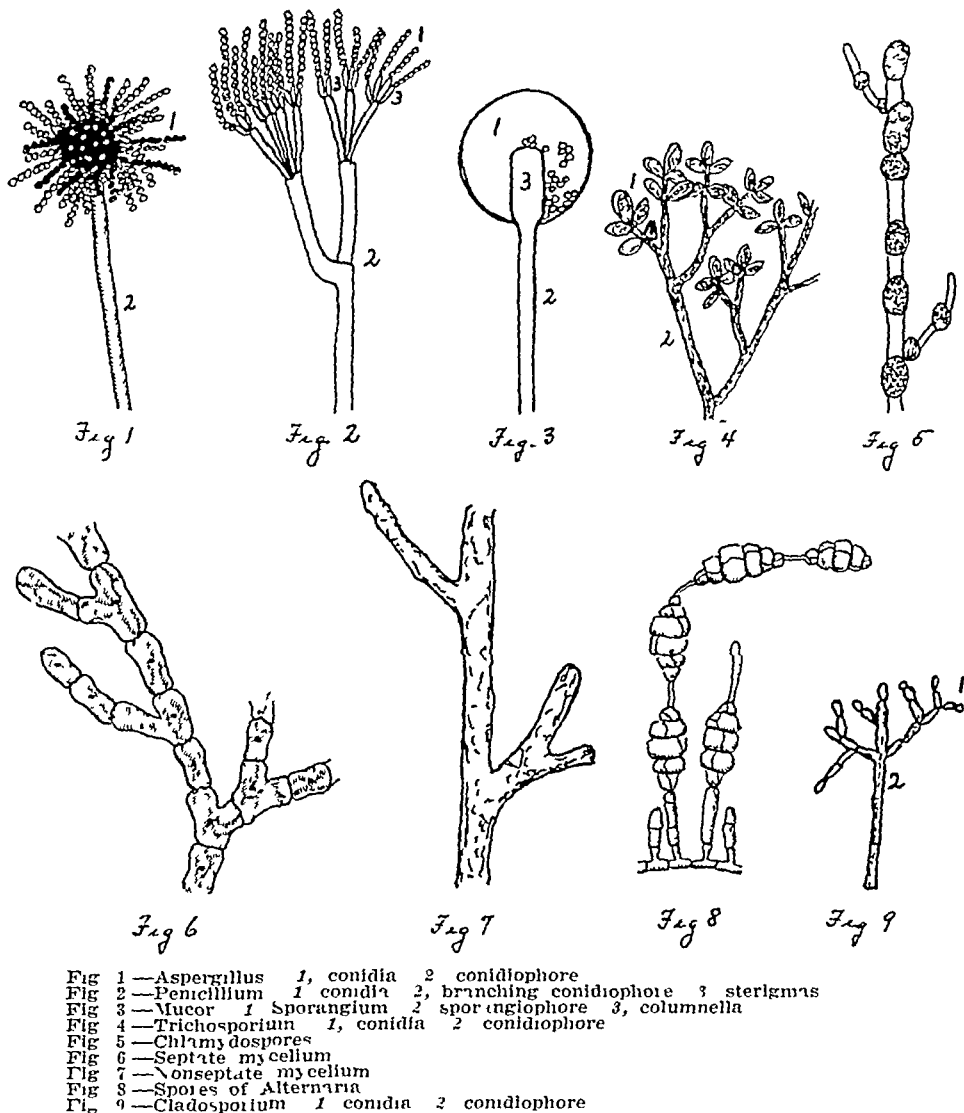
Trichosporosis This is due to a *Trichosporum*. Masses of mycelium grow as little hard knots on the hairs. These knots give off a rattling sound when the comb is run through the hair.

Blastomycosis Doubly contoured budding cells may be demonstrated in the pus either in potash solution or by staining. Histologic sections stained with eosin and hematoxylin will reveal the causative organism in the milium abscesses (Fig. 15). In some cases it may be necessary to make a number of sections, ten or more, to demonstrate the causative organism, in other cases each section may contain large numbers of the parasite. The organism (*Oidium gilchristi*) may be grown on the common laboratory agars. It produces a white fluffy growth made of mycelial threads (Fig. 14).

Pityriasis Versicolor Scrapings of the skin are placed on a slide, potash solution or sodium sulphide solution added and a cover slip laid on top of the liquid. After a few minutes with the sulphide solution (longer with the potash) the clusters of spores and the short mycelial threads will be seen (Fig. 11).

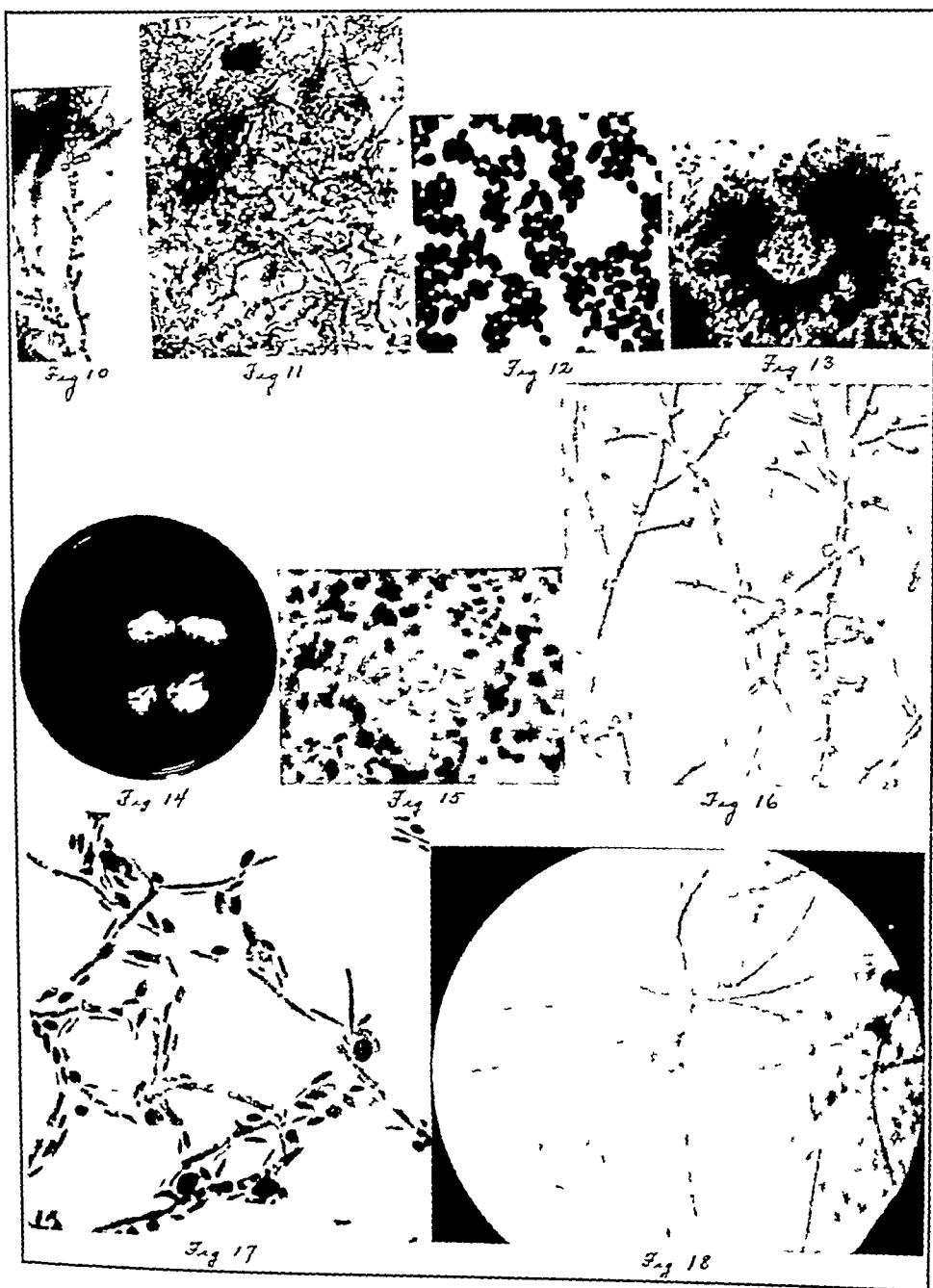
Trush Slide preparations from the patches stained by Gram's method will show gram-positive budding forms. If this material is plated the colonies may be isolated by identifying them by use of the 16 mm. objective of the microscope as described in a previous section of this paper. The colonies have a greenish cast, are somewhat circular and are composed of globular elements. A pure culture planted into dextrose broth after a few days show not only the budding types but also a mass of long branching mycelial threads, many of which have budding spores. To determine if these forms produce ascospores it will be necessary to plant some of the growth on a plaster of Paris block. This block is made by mixing plaster of Paris with water, pouring into a paper mold and allowing it to harden. When this has occurred, remove it from the mold

and place it in a deep Petri dish or test tube. Water or 0.1 per cent peptone solution is added to thoroughly moisten the block, but the solution must not cover the surface. The moistened block is then sterilized in the autoclave. Growth from a young culture is generously spread over the surface of the block. McKelvey's medium may be used in place of the plaster of Paris block. This is



made by adding plaster of Paris to carrot infusion agar, which is then tubed, autoclaved and slanted as with any other agar culture medium.

The above methods may be used for monilia in general. The yeast-like bodies will be seen on microscopic examination, the material may then be plated, the organisms isolated in pure culture and identified. So much controversy has arisen concerning the species of the genus *Monilia*, that it seems best not to include them in this paper.



- Fig. 10—*Monilia*. Pudding mycelium.
 Fig. 11—*Malassezia furfur* from *Urticaria versicolor*. Wet preparation showing mycelium and spores.
 Fig. 12—*Monilia*. Budding forms (blastospores). Stained preparation from agar slant.
 Fig. 13—*Actinomyces* in tissue. Stained.
 Fig. 14—*Oldium gilchristi*. Growth on agar.
 Fig. 15—*Oldium gilchristi* in tissue. Stained.
 Fig. 16—*Sporotrichum schenckii*. Growth on agar. Unstained. 4 mm objective and $\times 10$ ocular.
 Fig. 17—*Sporotrichum schenckii*. Stained preparation from agar slant.
 Fig. 18—*Trichophyton* in skin. High dry. Sodium hydroxide preparation. 4 mm objective and $\times 10$ ocular.

THE BRONCHOMYCOSIS

Diseases of the bronchi and lungs may be due to, or associated with, the following fungi. Those resembling yeasts i. e., the budding forms, *Coccidioides*, *Endomyces*, *Monilia* and *Saccharomyces*, those of the slender filamentous type, *Actinomyces*, *Anaeromyces* and *Vibrithrix*, those of the larger type, *Hemispora* and *Oidium*, those with characteristic fructifications, *Acladium*, *Acromoniella*, *Alternaria*, *Aspergillus*, *Mucor*, *Penicillium*, and *Sporothecium*.

The descriptions of the above genera follow.

Coccidioides. Asci present, vegetative cells form a well developed mycelium. The asci contain a large number of spores, characters intermediate between *Saccharomyces* and *Monilia*.

Endomyces. Mycelium well developed, budding forms present, asci four spored formed asexually, reproduction by external spores, ascospores and spores situated inside the mycelial tubes.

Saccharomyces. Vegetable cells globose ellipsoid, ovate, pear-shaped, etc., reproducing by budding and remaining attached in short, simple or branched pseudomycelial groups, which may later separate, asci globose, ellipsoid, or cylindric, 1 to 4 spored (typically 3 to 4 spored), single or in chains, ascospores globose to ellipsoid.

Anaeromyces. Anaerobic, gram-positive nonmotile, branching diphtheroid bacteria, colonies moist.

Vibrithrix. Mycelium polymorphic, bacillary, vibrio-like, spirillum-like and at times clubbed. Globose or pear-shaped bodies, which vary in size, may be present. Generally motile, gram-negative, generally aerobic and facultative anaerobic, a few are anaerobic, grows on common mediums.

Hemispora. Mycelium thin, hyaline, branched and septated. Some of the mycelial filaments show a cushion-like structure termed the protoconidium, which later becomes segmented. These segments are known as deuteroconidia.

Oidium. Forms in which the hyphae are often long and branched, terminating in spores known as Oidia, which result from the septation of the mycelium. Budding forms are at times included in this genus (Fig. 15).

Acladium. Accessory fructifications absent, hyphae abundant, more than 1 micron in diameter, the hyphae are pale, elongated, septate and branched, without true conidiophores, sporophores unbranched, sporogenous apparatus but little differentiated from the mycelium, pseudoconidia borne on the walls of the mycelium.

Acromoniella. Mycelium septate, abundant, a dark brown spore, 7.7 to 9.7 microns in diameter, terminal on each conidiophore.

Alternaria. Conidiophores in bundles, erect, short, conidia septate in two directions, conidia in chains (Fig. 8).

Aspergillus. Conidiophores unbranched, rise from an enlarged cell of the vegetative mycelium, terminal portion swollen, conidia borne on sterigmata (Fig. 1).

Mucor. Sporangiophores arising singly, without stolons, spores borne in sporangia, mycelium septate (Fig. 3).

Penicillium Conidia on distinct conidiophores which branch at the tip, conidia 1 celled, neither conidia nor hyphae smoky or dark in color when young (Fig 2)

Sporotrichum Mycelium septate and branched, conidia borne laterally or terminally from all parts of the mycelium, true conidiophores absent, colonies may be white or colored (Fig 16)

MYCOTIC DISEASES OF THE NERVOUS SYSTEM

Abscesses containing yeasts or yeast-like organisms have been found in the brain and spinal cord in patients with a generalized infection of these fungi. A number of cases have been reported of meningitis due to *Torula histolytica*. This organism appears in the tissues as yeast-like cells, surrounded by a thick capsule. In culture the growth resembles that of the yeasts macroscopically and microscopically. No asci are formed and no gas is produced from any of the carbohydrates ordinarily used for fermentation tests.

MYCOTIC DISEASES OF THE ORGANS OF SPECIAL SENSES

The following fungi have been reported as the etiologic factor in diseases of the eye: *Actinomyces*, *Blastomyces*, *Monilia*, *Oidium*, *Aspergillus*, *Penicillium*, *Sporotrichum* and *Glenospora*. The genus *Glenospora* is defined as mycelium composed of abundant hyphae, septate and branched, conidiophores absent, hyphae pale or dark, small aleurispores become dark and are borne on the sides and tips of the hyphae.

Aspergillus infection in man is commonly located in the ear. *Monilia*, *Mucor*, and *Saccharomyces* infections of the ear have been less frequently reported.

MYCOSES OF THE GENITOURINARY SYSTEM

Primary or secondary infections of the urethra in which a discharge is generally present may be due to one of the following: *Actinomyces*, *Monilia*, *Oidium*, *Torula*, or *Saccharomyces*.

The most common fungus associated with vaginitis is the genus *Monilia*. A number of cases of vaginitis due to this genus have recently been reported. Other genera reported are *Alternaria*, *Aspergillus*, *Cladosporium*, *Hemispora*, *Penicillium*, *Torula*, and *Vibrio*thrix. Most of the latter group may be mere saprophytes.

INTESTINAL MONILIASIS

Patients who harbor *Monilia* in their intestinal tracts are not uncommon. Sprue has been said to be due to this genus. The *Monilia* may be demonstrated very readily by planting the suspected material on dextrose-tartaric acid agar.

MYCOTIC DISEASES OF THE SKIN

Mycotic diseases of the skin may be divided into two groups: (1) Those which produce an inflammation due to the invasion of the tissues, and (2) those which produce no true infection but merely a saprophytic growth on the epidermis or hairs (*Malassezia* belongs to this group). The first group is known as the dermatophytes or dermophytes.

The dermatophytes are characterized by a branched, septate mycelium, usually producing two or three forms of conidia in cultures, arthrospores, aleuriospores and spindles (fuseau) are produced

The Dermatophytes may be classified as follows

A No conidia in cultures, *Endodermophyton*

B Conidia of one kind

1 Conidia simple, globose to subglobose

a Conidia in clusters, *Malassezia*

b Conidia borne at tip, *Montoyella*

c Conidia borne on the walls or sides, *Pinoyella*

2 Conidia fusiform and septate, *Epidermophyton*

C Conidia of two kinds

1 Aleuriospores and arthrospores present, *Trichophyton*

2 Aleuriospores and spindles present, *Microsporon*

3 Aleuriospores, arthrospores and hyphae with dichotomous subglobose or club shaped apical branches, *Achorion*

The above genera are summarized below

Montoyella The members of this genus have two kinds of mycelium, the one slender, branching and segmented, the other broader with numerous intercalary chlamydospores. From the broader filaments are borne delicate hyphae, which terminate in globose and pear-like conidia

Pinoyella Mycelial filaments and spores in the lesions, and conidia-bearing hyphae in cultures. The spores are situated on the walls or sides of the tubes. No perithecia or asci present

Malassezia In man mycelium broken into septate segments, with T-shaped or budding extremities. The hyphae bear round or oval conidia, which may be solitary or in clusters and may be smooth or with radial longitudinal or spiral marks. This organism has not been cultivated on artificial media (Fig 11)

Epidermophyton Mycelial filaments and spores present in the lesions and with pluriseptate spindles present in the cultures, hairs or hair follicles are not attacked, grows in the superficial layers of the epidermis. Spiral hyphae, which are present in most species of *Trichophyton*, are absent. There are no perithecia or asci present

Microsporon Members of this genus produce mycelial filaments and spores in the lesions. These spores are small, globose, and about 2 to 3 microns in diameter. In cultures hyphae-bearing sessile conidia and septate or nonseptate fusiform bodies may be seen. Perithecia and asci are absent

Trichophyton This genus is characterized by the formation of very distinct arthrospores in the mycelial filaments. These are present in the lesions and in cultures. There are no fusiform bodies present (Fig 18)

Achorion Mycelial filaments and spores are present in the lesions. In cultures, hyphae are present, which bear conidia laterally and at the tips. Fusiform bodies are also present in the cultures, these bodies are in the form of club-shaped terminations of the filaments. Asci and perithecia not present

Endodermophyton The members of this genus grow between the superficial and deep layers of the epidermis. Mycelial filaments and spores are pres-

ent in the lesions. No conidia are formed in cultures. No spindles, perithecia or asci present. To cultivate this fungus, treat the scales with alcohol for five to ten minutes, wash with sterile water and place one scale in each of several tubes of dextrose broth. Growth appears in about a week. In about three weeks the fungus may be transferred to dextrose agar slants.

CLASSIFICATION OF FUNGI

The classes of the subdivision Fungaceae which are of medical interest are the Phycomycetes, the Ascomycetes and the Fungi imperfecti.

The Phycomycetes produce a copious matted mycelium which is nonseptate, and reproduce asexually by means of sporangia borne on columnellae.

The subclass zygomycetes reproduce either asexually (production of sporangia) or by isogamy (two similar but sexually different cells conjugate which on fusion form a zygospore).

This subclass is divided into two orders. Those with several asexual spores on sporangia (Mucorales), and those with solitary spores (Entomophthorales).

The order Mucorales contains the family Mucoraceae. In this family are three genera: Mucor, without rhizoids, Rhizomucor with rhizoids and unbranched aerial hyphae and Rhizomucor, with rhizoids and ramified mycelium.

The class Ascomycetes contains the following genera: Saccharomyces, spores smooth, spore membrane single, copulative process absent, rudimentary mycelium present with transverse septation, alcoholic fermentation produced. Endomyces, mycelium well developed, ramified or not, simple or septate, budding cells present, asci four spored.

KEY TO SOME OF THE GENERA OF FUNGI

- A Mycelium white or bright colored, conidia 1 celled hyaline or bright colored, globose to ovoid or cylindric
 - 1 Hyphae very short or obsolete, or little different from the conidia
 - a Conidia in chains produced by blastospores arising on the hyphae, globose or elliptic, hyphae branched MONILIA
 - b Conidia in chains, produced by arthrospores, ovoid to elliptic, hyphae branched OIDIUM
 - 2 Hyphae elongate and distinct from the conidia
 - a Conidia borne in heads
 - (1) Conidiophores inflated at apex, conidia in chains at the tip, globose to ellipsoid ASPERGILLUS
 - (2) Conidiophores not or but slightly inflated, conidia not in mucus, globose, in whorls at the tip, whorls unequal PENICILLIUM
 - b Conidia borne more or less irregularly on simple or branched but not inflated or whorled hyphae, conidia smooth or scarcely roughened
 - (1) Conidia borne on the walls or sides of the mycelium, conidiophores simple or nearly so, conidia globose to ellipsoid ACLADIUM
 - (2) Conidia borne at the tip and sides of the mycelium, conidiophores vaguely branched and of one kind, conidia globose to ellipsoid SPOTOPHICUM
- B Mycelium dark or smoky
 - 1 Conidia 1 celled dark or sometimes hyaline but the hyphae then dark, globose to oblong
 - a Hyphae very short or scarcely different from the conidia, conidial chains breaking up readily TOPULIA
 - b Hyphae manifest and distinct from the conidia

- (1) Conidia in chains on the branches, ovoid or globose, conidiophores erect, dendritically branched and septate, not spirally twisted *HORMODENDRUM*
- (2) Conidia not in chains
 - (a) Conidiophores branched, all hyphae more or less creeping, conidia smooth and sessile *TRICHOSPORUM*
 - (b) Conidiophores swollen at tip, conidia 4-8 superimposed, warted *HEMISPORA*
 - (c) Conidiophores not swollen at tip
 - ✓ Conidia solitary, smooth, on short lateral branches, hyphae forming a crust *GLENOSPORA*
 - ✓ Conidia solitary, smooth, terminal, sterile hyphae present, without bristles *ACREMONIELLA*
- 2 Conidia 2 celled, conidiophores definitely differentiated, conidia not in terminal heads, conidia in chains and of one kind, hyphae not inflated *CLADOSPORIUM*
- 3 Conidia many celled, with both longitudinal and transverse septa *ALTERNARIA*

In conclusion the reader is referred to the works of Henrici, Clements and Shear, Brumpt, Saito and Castellani for more extensive information

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BACTERIOLOGY OF PUS*

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PUS is formed as the result of an accumulation of leucocytes, largely the neutrophils, which have migrated from the blood vessels into irritated tissue. Necrosis and liquefaction of the cells usually follows. A positive chemotactic action is responsible for the accumulation of leucocytes. This chemotaxis is due to the presence of cellular constituents, possibly protein in nature, which have been set free by injured cells. These may be the fixed tissue cells of the body or may be of microbial origin. Pus does not therefore, necessarily contain bacteria but in an overwhelming proportion of instances in which this material is sent to the laboratory for examination, bacteria of some variety are found. It may however be difficult to demonstrate the presence of bacteria in pus from the cases of certain diseases after therapeutic measures have been instituted.

The variety of bacteria encountered in pus, is great. Indeed, a complete discussion would entail the preparation of a textbook on pathogenic bacteria. It is, therefore, possible to give here only an outline of methods in most common use, and to refer to the microorganisms most generally encountered. Regardless of the source of material, whether from a running ear, a case of empyema, furunculosis, or meningitis, certain definite procedures can usually be followed with the introduction of proper variations dependent on the results obtained as the laboratory worker proceeds. It is possible to give in this brief discussion, only the most outstanding characteristics of the groups of bacteria referred to,

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these characteristics being chosen because of their value to the clinical laboratory. For exact identification of bacterial species reference must be made to the literature.

The proper collection of material is essential for a satisfactory laboratory examination. Skin contaminants must be rigidly excluded. Too great emphasis cannot be placed upon this point if the laboratory worker is to give the clinician a true picture of the bacteriology of the material submitted.

The first step in laboratory examination of pus is under all conditions the *preparation and examination of a proper smear* on a microscope slide. Care should be taken to make thin film preparations. A stain according to Gram's method gives the most valuable indication of any single stain but will not, of course, detect the tubercle bacillus. Hence it is extremely desirable for the laboratory to be furnished with accurate information concerning the source of the material. Various modifications of the original Gram method have been introduced.¹ Our experience has been that careful attention to the details of the technique is fully as valuable in yielding consistent results as any modification of the solutions employed. This is particularly true in regard to the alcohol (or acetone) decolorization. In "old" pus the Gram stain may be uncertain. In many laboratories the methylene blue stain is used for the detection of gonococci. While this is reasonable under special conditions where the clinician and the laboratory work in close cooperation it is not to be recommended. Furthermore it is highly desirable to make a Gram stain if organisms resembling the gonococci are seen in the material submitted so little is gained by the use of methylene blue. For pus from tubercular conditions, the Ziehl-Neelsen method, or some of its modifications is in general use. The methylene blue stain may be used for detecting organisms resembling diphtheria bacilli while either methylene blue or gentian violet are useful for staining the spirochetes and fusiform bacilli characteristic of Vincent's infection.

It is undesirable that a laboratory report based upon the microscopic examination of a direct smear should record the name of the suspected organism. A possible exception to this is in the examination for tubercle bacilli and here the exception can be made only in material which is not likely to contain other "acid resisting" organisms. Undoubtedly, in many instances the name of a bacterium recorded on a laboratory report as a result of direct examination is correct, as for example in vaginal or urethral smears in gonorrhea, in spinal fluid pus in epidemic meningitis in staphylococcus infections, and in Vincent's angina. However so many exceptions occur that such reports can be regarded only as preliminary and unscientific. Organisms of the *N. catarrhalis* group may be present in supposedly gonorrheal pus or gonococci or *N. catarrhalis* might be the cause of meningitis, although it must be admitted that this difficulty rarely occurs.

It is sometimes claimed that the pneumococcus may be distinguished from *Streptococcus viridans* by direct examination. We do not believe that this can be done. Indeed it should never be attempted. The streptococci are highly pleomorphic and oftentimes closely resemble the typical lanceolate pneumococci. With the present possible use of antipneumococcal serums in Type I and II infections it is all the more necessary not to hazard a guess on the basis of micro-

scopic appearance Morphologic variations in bacterial species have been definitely established Residence in body tissue, living or necrotic, may accentuate these changes In a fairly large series of cases of influenzal meningitis that have come to our attention, the organisms usually appeared in the spinal fluid as tiny gram-negative diplococci with only an occasional rod In a few instances, rods predominated and in one case swollen cocci and rods were found The laboratory should report the result of the Gram stain as quickly as possible, together with a statement concerning the appearance of the organisms found In many cases this description will be sufficient for the purposes of the clinician

Satisfactory identification of bacteria found in pus, can be made only as the result of biochemical and serologic studies The isolation of the organism in pure culture is of course, the first necessity The culture medium of choice will depend to some extent on microscopic findings It is impossible to pick one formula from the many hundreds available for media, and recommend it as the best under all circumstances For all around work, except in cultivating tubercle bacilli and anaerobes, a meat infusion peptone agar containing one-half to one per cent of dextrose with a reaction of P_H 7.6 and containing about 5 per cent of defibrinated blood (the source of blood is in most instances immaterial) is to be recommended This medium, in Petri dishes, will grow all the ordinary pathogenic bacteria encountered in pus except as just noted Better results are probably obtained if hormone broth is used as the basis of the medium but our experience has been that the extra work involved is not justified by the results The medium is the one commonly recommended for culturing of meningococci from the throat Our experience has been that it is excellent for the isolation of gonococci and that *H. influenzae* and *H. pertussis*, while not developing so rapidly as on some other media, can be grown satisfactorily The main disadvantage of the medium is that some strains of streptococci fail to show as early hemolysis as when a more acid medium is used If, therefore, the laboratory worker is certain that he is dealing with the streptococcus-pneumococcus group, the ordinary blood agar medium (P_H 6.8) gives earlier differentiation

While certain differentiations can be made by the use of the ordinary so-called plain agar medium, such as between *N. catarrhalis* and the pharyngis siccus group from the gonococcus and the meningococcus, other media are of far more use in the clinical laboratory Where material is from such a source that it is unlikely to contain more than one organism, semisolid agar will give excellent results It does not however, give any species differentiation such as is possible by the use of the blood agar plate above described Many other special media are in use Tubercle bacilli may be grown on a variety of media When the organisms have been found in the direct microscopic examination, cultivation is not usually necessary unless it is desired to make a complete bacteriologic study of the case It very frequently happens however, that the number of tubercle bacilli present in the material is so small that they cannot be found in the direct smear Where there is any suspicion that these organisms might be present either guinea pig inoculation or culturing should be resorted to Again the medium to be used will depend somewhat upon the experience and ideas of the laboratory worker Cooper's medium, Petrioff's medium Petragagni's medium, Sweeney's medium and others have been found useful In our opinion,

the preparation of the specimen is of equal importance with the medium. Other bacteria must be eliminated preferably by the use of acid (specimen and 3 per cent hydrochloric acid in equal volumes has proved successful in our hands), and more than one tube must be inoculated. If the guinea pig method is used, not less than two pigs are required.

Even if no bacteria are visible in the direct microscopic examination it is still necessary to culture the pus. Either the blood agar plate above described infusion broth or a semisolid medium may be used. Anaerobic methods should also be employed if gangrene or tetanus is suspected. Brain broth or deep glucose agar are excellent general media for anaerobic work. A great variety of media are recommended to cover special circumstances. All cultures should be incubated for not less than four days provided no growth appears within that time. Many mistakes are made in the identification of bacteria by the use of insufficient incubation periods. This is particularly true in testing the fermentation reactions of pure cultures. Delayed fermentation of carbohydrates as well as delayed reaction in milk are more commonly encountered than is sometimes supposed.

As indicated above it will be possible to mention only the most commonly encountered pathogenic bacteria and to give only brief descriptions of the various species. It is hoped to make these descriptions sufficiently adequate to cover the needs of most clinical laboratories. They are, of course quite insufficient for scientific purposes or for detailed study of clinical cases. It is to be regretted that in so many instances clinical necessity is a bar to accurate scientific work, but it must be admitted that with few exceptions detailed laboratory examinations of bacterial cultures from pus do not give the clinician a better basis for therapeutic measures than a less detailed study. There are, however some exceptions to this point of view. An attempt has been made to list in connection with the brief descriptions of bacteria, the source of material most commonly yielding the species described. It should be emphasized that the statements that follow refer only to pus.

The staphylococci are more frequently found in pus than any other group of bacteria. Among these, *Staphylococcus aureus* predominates. This is a gram-positive organism appearing in characteristic clusters on the microscopic slide. Irregular forms are sometimes encountered in pus. The organisms grow readily on all the common culture media and produce a golden yellow pigment. Dextrose is always fermented with production of acid but no gas. Maltose, saccharose, and lactose are usually attacked. Acid is produced in milk. Gelatin is usually liquefied. On blood agar many strains give a clear zone of hemolysis. Immunologically, the group is heterogeneous.³ No group of bacteria with the possible exception of the streptococci is found in so many pathologic conditions in which pus is present. Skin conditions of great variety, including furunculosis and impetigo, subcutaneous abscesses, abscesses of various internal organisms, middle ear infections, osteomyelitis and meningitis are examples. They also commonly occur in mixed infections.

Streptococci are nearly as frequently encountered in pus as are staphylococci. They are gram-positive organisms occurring in pairs or chains usually nearly spherical but not infrequently elongated. Certain strains have been

shown to be highly pleomorphic.⁴ This group of bacteria are most easily isolated on blood agar. Various degrees of hemolytic activity are observed on this medium.⁵ For practical purposes two types must be noted, those that produce a clear zone and those that produce a green coloration. Fermentation reactions are quite varied. Dextrose is always fermented; most human types ferment lactose, while various reactions are obtained with mannite, inulin, and other carbohydrates. Gelatin is not usually liquefied. Any classification on the basis of fermentation reactions is of little clinical significance.

The *hemolytic streptococci* are the more important of the two groups as differentiated on blood agar, because of their greater pathogenic power. No practical laboratory tests have been devised for distinguishing the varieties of these hemolytic organisms in relation to their specific pathogenicity, although many attempts have been made to do so. Neither serologic^{6, 7, 8} including agglutination, complement fixation or opsonic, reactions nor cultural^{9, 10} tests have proved of value. This group is found frequently in abscesses occurring as complications of scarlet fever or other streptococcus infections, in ear infections, in meningitis, in bone infections, in tonsillar abscesses, in empyema, etc. The "green producing" streptococci are less frequently encountered. These organisms must be differentiated from the pneumococci (see below).

Pneumococci are frequently present in pus. These are gram-positive cocci usually seen in pairs but sometimes in short chains. The typical cells are elongated. It should be noted however, that in the examination of material from the body, atypical morphologic forms are more likely to be seen than the typical forms observed in pure cultures. Indeed, this is true of all varieties of bacteria. Pneumococci are easily isolated by mouse inoculation, in Avery's broth or on blood agar and produce a green coloration. The biochemical reactions are similar to those of the streptococci and are of little value in the clinical laboratory. The differentiation between a pneumococcus and the "green producing" streptococcus is important. In our opinion, neither colony characteristics nor morphology are sufficient for this purpose. The use of the agglutination or precipitin test combined with solubility in bile, are required. Solubility in bile is the generally recognized criterion for the separation of pneumococci and streptococci. However, we have isolated organisms of the pneumococcus streptococcus group that are agglutinated by a pneumococcus type serum but failed to dissolve in bile. For this reason, reliance should not be placed solely on the bile test. Pneumococci are encountered in pus from ear infections, from meningitis, from peritonitis, in lung abscesses, etc. The typing of pneumococci may be important in cases where the clinician desires to use specific antipneumococcic serum.

Meningococci may be found in pus present in the spinal canal. They may also be present in the nasopharynx and the blood, but a discussion of these possibilities is outside the province of this paper. The meningococci are gram-negative biscuit shaped diplococci. While they are reported to be characteristically found in the leucocytes their presence outside of these cells should not be disregarded. Indeed, the extracellular position is characteristic of material from fulminating cases. Isolation may be made on the special blood agar medium already described or in semisolid agar.¹¹ It is important to work with a fresh specimen. Isolation after intraspinal injection of antimeningococcic serum is

difficult, although a stained preparation may reveal the organisms. Meningococci ferment dextrose and maltose. Fermentation reactions may be used to differentiate from other morphologically similar gram-negative cocci. For practical purposes however identification of the meningococci can be more quickly made by the use of a polyvalent antiserum. Therapeutic serums with a relatively high agglutinin titer may be used for this purpose but it should be noted that odd strains are sometimes encountered. There is no practical value in grouping the meningococci at the present time but this statement may not hold when more knowledge is obtained concerning specific therapeutic serums.

Pus containing *gonococci* comes as a rule, either from the genital tract or the eyes. The organisms are infrequently encountered in spinal and other body fluids or organs. They are biscuit shaped, gram-negative, usually intracellular, and cannot be distinguished with certainty on purely morphologic grounds from other gram-negative diplococci. However if the microscopic laboratory findings correlate with those of the clinician, it is unnecessary to proceed with further identification. In certain cases complete laboratory identification may be extremely desirable. A great variety of media have been suggested for isolation of the gonococcus. The medium should be slightly alkaline and contain body fluids such as ascites, blood serum, or defibrinated blood. We have found the blood agar used for meningococcus work to be quite satisfactory. Gonococci ferment dextrose, but not maltose. The colony appearance on blood agar plates serves to differentiate them from many of the gram-negative diplococci. In order to obtain a successful isolation, fresh material must be used.

N. catarrhalis and the *chromogenic diplococci* are common inhabitants of the upper respiratory tract. They are infrequently associated with pyogenic conditions. *N. catarrhalis* can be distinguished from the meningococci by its ability to grow on an ordinary plain agar medium and its inability to ferment carbohydrates. The chromogenic diplococci such as *N. pharyngitidis* etc. have stronger fermenting powers than the meningococci or gonococci. The extent to which the members of these two groups of organisms are concerned with pathogenic processes is problematical.

Members of the *colon typhoid group* are sometimes found in pus. *B. coli* being the most frequently encountered. As a group, they are relatively easy to identify. The gram-negative rods grow on plain agar and on various special media such as that devised by Endo. Gelatin is not liquefied. Fermenting properties vary. Members of the colon subgroup produce acid and gas from dextrose and lactose and sometimes from saccharose, the salmonella or paratyphoid group ferment dextrose with acid and gas production, but not lactose, the typhoid dysentery group ferment dextrose with the production of acid only. Colonies of *B. coli* of intestinal origin have a characteristic appearance on such media as Endo and eosin methylene blue. The other subgroups give a bluish white colony. The group as a whole is too well-known to require description here particularly as the paratyphoid and typhoid subgroups are infrequently found in pus. Where possible, serologic identification should be made including agglutinin absorption tests in the salmonella group. This last includes quite a variety of bacterial species and many groups when studied on the basis of cultural and serologic properties.

The *Proteus* group is sometimes found in urinary pus. These organisms may also be associated with pyogenic processes other than those found in the urinary tract either in association with other organisms or as the supposed etiologic factor. The organisms are gram-negative pleomorphic bacilli usually motile and do not ferment lactose but produce acid and gas from dextrose and saccharose. Most of them liquefy gelatin. Serologically, the proteus group is heterogeneous.

Considerable interest has been aroused during the past fifteen years over the disease known as *tularemia*. It is probable that the first human cases were eye infections reported by Wherry and Lamb.¹² *B. tularensis* is rather difficult to grow, requiring either a medium containing egg yolk or blood or blood serum preferably in the presence of tissue, such carbohydrates as glucose, levulose, mannose, and glycerin fermenting with the production of acid. Final identification should be made by means of the agglutination reaction.

Bacillus pyocyaneus is found in so-called "blue pus" particularly from surgical dressings. Its virulence toward man is relatively low, apparently being somewhat greater for children than for adults. The bacillus is gram-negative, actively motile, grows well on plain agar, produces little or no acid from dextrose, liquefies gelatin and digests milk. It produces a blue-green pigment and a lysis toward red blood cells. It has been reported as one of the causes of middle ear infections and meningitis.

The *Friedlander bacilli*, while closely resembling *B. aerogenes* in cultural reactions can be distinguished by their morphology, by the presence of capsules and the mucoid growth on an agar medium. The organism is more frequently found in pus from upper respiratory tract infections than from any other type of disease although they have been reported in empyema, meningitis, cystitis, etc.

Organisms of the *diphtheria* group and the diphtheroids are rather frequently encountered in pus. However, they appear to be most often present as secondary invaders. In the latter case, either the diphtheroids or the very short bipolar staining organisms (similar to Wilson's Type D) are more likely to be found than are the morphologic types usually associated with diphtheritic infections (Wilson's Type C). Organisms of the diphtheria group present as secondary invaders are usually avirulent but there are exceptions to this statement. Their occurrence as the primary cause of pus-producing infections cannot be lost sight of. Ears, thus infected and draining, may serve as foci for dissemination of the disease. Pleomorphism in the diphtheria group is too complex a subject to discuss here. Biochemically, the strains encountered are not particularly active. The true diphtheria bacillus produces acid from dextrose, galactose, and levulose and either no reaction or a small amount of alkali in milk. The pseudodiphtheria bacilli have a variety of biochemical reactions but in general the differences from the toxin-producing organism are relatively small.

In culturing pus, the possible presence of *anaerobes* must not be overlooked. The tetanus bacillus is an example. In most instances relatively simple anaerobic methods are just as effective as are the more complicated ones. Growth will usually occur in a deep glucose agar shake culture or even in flasks containing broth. Probably the best medium for general use is one containing tissue, prefer-

ably brain¹³ B tetanae belongs to the group of anaerobes which do not attack carbohydrates. Gelatin and coagulated protein such as blood serum, are usually slowly liquefied. A soluble toxin of high potency toward guinea pigs may be produced. Spores are formed usually near one end of a cell giving a typical appearance resembling dumb bells.

The above mentioned groups of bacteria represent those most frequently encountered in clinical laboratory work but as stated earlier in this paper most any pathogenic bacterium may be found in pus. Attention should be called to the hemophilic pasteurella and lactobacillus groups. No mention has been made of material from pustules such as those found in smallpox. The possible presence of a filterable virus as the etiologic factor must always be considered even if known bacterial forms are present. Such pus, however rarely finds its way to the clinical laboratory perhaps not as often as it should. At any rate the laboratory worker examining pus should not have any preconceived notions as to what he will find. Mixed cultures occur frequently and it is sometimes difficult to determine whether the localized infection is due to a mixture or to only one of the bacteria isolated. Even detailed studies do not always lead to a satisfactory conclusion concerning the etiologic agent.

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BACTERIOLOGIC EXAMINATION OF BLOOD AND SPINAL FLUID*

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THE problems of the clinical bacteriologist have been complicated in recent years by the introduction of a great variety of methods and mediums. Research has also tended to shake our faith in the fixity of bacteriologic types and suggests that the life cycle of microorganisms is not as simple as previously supposed. Regardless of these uncertainties the clinician is depending more and more on laboratory findings. A discussion of practical bacteriologic methods for the examination of blood and spinal fluid is here presented.

BLOOD

Collection of Specimen—The sample is usually obtained from a vein at the bend of the elbow by means of a syringe and needle or such special apparatus as the Keidel vacuum tube. The latter method is especially useful for the private practitioner who does not have adequate facilities for sterilizing apparatus and where the transportation of the specimen by mail or messenger is necessary. For the hospital laboratory, however, the syringe and needle method is preferable.

The skin of the patient is sterilized with two coats of tincture of iodine and one coat of 95 per cent alcohol. Each coat of iodine should be permitted to dry before the application of the next coat.

Sterilization of Syringes and Needles—As the sterilization and care of syringes and needles are usually inadequate the following satisfactory method is given in detail.

The barrel of the syringe, the piston and two needles are each placed in a pyrex glass tube closed at one end and open at the other. The tubes should be just large enough for the parts to be removed easily, for the larger syringes, they will have to be especially made. Cotton is placed in the bottom of each tube to prevent breakage, and after the parts have been inserted the open ends are stoppered with cotton plugs. The tubes are then tied together, wrapped in paper and retied, and sterilized in the dry sterilizer at 150° C for two hours.

At the bedside, the instrument can be assembled easily with little risk of contamination. The cotton stopper in the tube containing the piston is first removed and if the latter has been encased properly, the head will easily slip into the hand. The stopper of the tube containing the barrel is next removed, the piston is slipped into the barrel and the two withdrawn together. In the same manner a needle can be attached to the tip of the syringe and after tightening the needle it is ready for use. If for any reason the blood cannot be taken immediately, the syringe can be replaced in one of the tubes with a minimal risk of contamination. After the blood has been taken the syringe and needle should be washed in water before clotting of the residual blood takes

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place. Drying is quickly accomplished with alcohol and ether. The needle should be sharpened and tested for flaws. After determining that syringe and needles are in perfect condition they may be rewrapped and sterilized.

There are several advantages in this method. (a) The syringes and needles are actually sterile and dry. (Boiling for fifteen minutes or longer will not kill some contaminants.) (b) They can be readily transported in the sterile state. (c) They will remain sterile indefinitely and will always be on hand for immediate use. (d) The possibility of contaminating the apparatus at the bedside is reduced to a minimum. (e) Both syringes and needles prepared in this way will outlive many times similar equipment subjected to frequent boiling.

Routine Cultures—Two mediums of special importance for routine culturing of blood are beef heart infusion broth and beef heart infusion agar. The details of their preparation are given in the article on the "Technic for the Isolation of Streptococci." The broth is made up in bottles or flasks, each containing about 50 c.c. The agar medium is conveniently placed in test tubes, 9 c.c. to each tube. Prior to use the agar is heated in a water-bath until melted and allowed to cool in the air to about 40°C , 1 c.c. of the patient's blood is then added, whirled until mixed, and poured into Petri dishes. The blood agar plate has several advantages. It gives a quantitative estimate of the number of bacteria in the circulating blood. Also it frequently permits an early laboratory diagnosis. It is always well to make a culture in nutrient broth as organisms that do not appear on the plates occasionally grow in this medium. In both cases, the amount of blood compared to the amount of medium should not exceed the ratio of 1 to 10. The optimum temperature for growth is 37°C and the hydrogen ion concentration is slightly alkaline with a P_H of 7.4 to 7.6. It is usually advisable to incubate some of the cultures under anaerobic or partially anaerobic conditions as certain bacteria grow more luxuriantly in the absence of oxygen.

The blood agar plates and blood broth tubes should be observed daily for evidences of growth. As soon as the blood broth becomes cloudy, smears stained by Gram's method or with methylene blue should be made, as well as subcultures on blood agar plates and in blood broth tubes. Blood cultures should be observed for at least ten days and preferably fifteen to thirty days before being considered negative. There is a marked tendency to discard cultures at too early a date.

Gram-positive Cocci—Staphylococci grow readily in all the usual laboratory mediums but for purposes of study, culturing on blood agar pour plates and in blood broth are the most satisfactory. In examining cultures attention should be given to their hemolyzing properties and their ability to produce pigment on solid mediums. Staphylococci, especially of the white variety, are occasionally seen as contaminants in blood culture work and a few colonies present on one plate and absent on others should make one suspicious of contamination. Staphylococci are identified by the characteristic appearance of their colonies on blood agar plates, by the fact that they are gram-positive and by their tendency to form clumps instead of chains. Examination of smears for morphologic characteristics should be made from cultures in liquid mediums.

Streptococci may be recovered in blood cultures from patients suffering from a great diversity of diseased conditions. From foci of infection in the skin, tonsils, teeth, sinuses, and the middle ear or from infected wounds, streptococci may invade the blood stream, producing a rapidly fatal septicemia, or chronic conditions, such as pleurisy, pericarditis, endocarditis, or arthritis. Primary cultures of streptococci grow delicately on artificial mediums and considerable care must be exercised both in obtaining them and in keeping them alive. The importance of observing blood cultures in liquid mediums over a long period is indicated by the positive results that have been reported after an incubation period of thirty days or more. Streptococci are differentiated from staphylococci by their characteristic growth in blood agar pour plates and their tendency to form long or short chains. Generally the individual cocci are smaller in streptococcal than in staphylococcal cultures.

Pneumococci are present in the peripheral blood of a large proportion of patients with lobar pneumonia and in the blood of some with bronchopneumonia. Positive cultures of this organism are sometimes obtained in complications of pneumonia, such as empyema, meningitis, mastoiditis, endocarditis, and pericarditis. The pneumococcus grows delicately in primary cultures but readily adapts itself to artificial mediums. Cultures are made in nutrient broth and in blood agar pour plates. Pneumococci are differentiated from streptococci by the bile solubility test, the former being bile soluble and the latter, bile insoluble.

Gram-negative Cocci—Both the gonococcus and the meningococcus are occasionally recovered from blood cultures. Gonorrhea is sometimes complicated with septicemia and endocarditis, and in such cases the gonococcus is usually recoverable from the peripheral circulation. In like manner, the meningococcus, in cases of cerebrospinal fever, sometimes invades the blood stream. A meningococcal bacteremia is also possible without symptoms of meningitis.

Both organisms are quite fastidious in their nutritive requirement and grow poorly, or not at all, in the usual laboratory mediums, those containing albuminous material seem to be necessary for their successful cultivation. Beef heart infusion agar, or "hormone" agar to which hydrocele or ascitic fluid has been added, in the proportion of two parts of agar to one part of fluid is a very satisfactory medium. Pour plates containing 9 c.c. of medium for each cubic centimeter of blood give the best results. In culturing meningococci in agar medium it is important that the agar be cooled to at least 40° C. before adding the blood, as meningococci are killed at 41° C. Both meningococci and gonococci are very sensitive to loss of moisture. If the water of condensation is not present, the cultures may be placed in a closed jar containing moist cotton or filter paper. Gonococci, in addition, may be cultured in beef heart infusion broth containing hydrocele or ascitic fluid in similar proportions. The optimum incubation temperature for the gram-negative cocci is about 37° C. Differentiation of meningococci and gonococci is discussed in the section on spinal fluids.

Typhoid-Colon Group—The most favorable time for making blood cultures on patients with typhoid fever is during the first week of the disease. While the typhoid bacillus grows readily in nutrient broth, provided the P_H is from 6.8 to 7.0, the most satisfactory medium is ox bile, either alone or containing a small amount of glycerin and peptone. Test tubes containing from 7 to

10 c.c. of this medium are made up and autoclaved. It is advisable to add the blood in amounts varying from 0.5 to 3 c.c. to several tubes of bile medium. The cultures are incubated at 37° C. and subcultured every twenty-four hours on lactose litmus agar or Endo's medium. Positive results are usually obtained in seventy-two hours but may be delayed as long as five or six days. Organisms of this group are identified by their cultural characteristics, by their fermentative activities, and by agglutination tests.

It is convenient in private practice to place the bile in sterile vaccine bottles into which the patient's blood is injected. In this way specimens can be readily transported to the laboratory.

Influenza Group—The difficulties experienced by pioneer workers in isolating organisms of this group have been lessened by more efficient methods of cultivation. Suitable mediums consist of beef heart infusion broth or agar containing blood subjected to tryptic or peptic digestion. Chocolate agar, prepared by adding defibrinated blood to agar medium which has been heated to 100° C. is of value.

The influenza bacilli are strictly aerobic. shallow layers must, therefore, be employed in liquid medium. If a solid medium is used, the blood should be smeared lightly over the surface. Colonies of these organisms appear as circular, colorless, translucent discs, with sharp edges and fine granulations in the center. The organisms die easily—rarely can they be kept longer than ten days. On smears the organisms appear as minute coccobacilli measuring from 0.2 to 0.8 μ , are gram-negative and stain faintly, on ordinary agar they grow as very small pinpoint colonies barely visible to the naked eye.

Brucella Group—Members of this group may be recovered from the peripheral blood in a high percentage of cases of undulant fever. The blood is best taken at the height of an exacerbation, although organisms are occasionally recovered during afebrile periods. It may be inoculated in either liquid or solid mediums. The organisms will grow in simple nutrient broth, provided the blood is in the proportion of at least one volume of blood to eight of medium. Trypsinized broth and liver infusion broth (Stafseth's broth) have been employed with success. With liver infusion agar and glucose agar, which are sometimes used, the blood is spread directly on the surface of the plates. The most favorable temperature for incubation is 37° C. and the organisms grow best in an alkaline medium with a P_H of 7.4. Growth generally appears in from three to four days but may be delayed as long as two weeks.

The *Bacillus abortus* of bovine or porcine origin generally requires an environment containing a high percentage of $C O_2$ for growth.

The laboratory diagnosis of *Brucella* infections is made by isolating the organisms from the blood by demonstrating agglutinins in the patient's serum, and by testing for skin sensitivity.

SPINAL FLUID

Collection of Specimen—The spinal fluid may be conveniently collected in centrifuge or test tubes. Part of the material should be set aside for cell count, chemical tests and complement fixation. The material for culture especially when meningococci and gonococci are suspected should be kept at body tempera-

ture by means of a water jacket and sent immediately to the laboratory for culturing. Part of the specimen may be set aside for guinea pig inoculation.

Microscopic Examination of Films—If tuberculous meningitis is suspected, part of the fluid should be permitted to stand until a coagulum forms. This fibrin clot is then gently floated out on a slide, dried, fixed, stained according to Ziehl-Neelsen technique, and examined for tubercle bacilli. Great care must be exercised in withdrawing the clot, as touching it with a platinum loop usually causes it to form a tough mass. Examination of the pellicle is more frequently successful than examination of the centrifuged specimen.

The remainder of the spinal fluid is centrifuged for one-half hour. The supernatant fluid is poured off and smears are made of the sediment. It is well to stain slides according to the Ziehl-Neelsen technique, with Gram's stain, and with methylene blue. From the smear stained with methylene blue an estimate of the relative number of each type of leucocyte can be made.

The following organisms are differentiated by Gram's stain

- 1 Gram-positive streptococci, pneumococci, staphylococci
- 2 Gram-negative meningococci gonococci, Pfeiffer's bacilli

If clinical findings are taken into consideration, a tentative diagnosis can frequently be made from the examination of smears. In the case of finding tubercle bacilli, the diagnosis is definite.

As the patient's life may depend on the rapid diagnosis of meningococci in the spinal fluid, it is always well to study the smears very carefully when gram-negative cocci are found. The textbook picture of stained smears of the cerebrospinal fluid showing large numbers of kidney shaped cocci arranged in pairs with the indented surfaces opposed to one another is seldom seen in the clinical laboratory. There is a great diversity in the microscopic appearance of films of the spinal fluid from patients with meningococcal meningitis. The cocci may be few or numerous, largely intracellular or extracellular. They may be in pairs or tetrads, but often are found singly. It is interesting to note that when meningococci are found singly they are usually round or elliptical, seldom giving the typical flat kidney shape picture. When rapid diagnosis is requested, a positive report for meningococci may be made whenever gram-negative cocci are found in the spinal fluid. The administration of antimeningococcal serum is justified even when organisms are not found in the smears, provided the leucocyte cell count is high and the smear demonstrates that this increase is largely due to polymorphonuclear cells. Of course, it is advisable in such instances to take into consideration the patient's clinical picture.

Cultural Examination—The diagnosis of tubercular involvement of the meninges is frequently made from careful examination of smears only, however, if tuberculosis is suspected and direct microscopic examination has failed to demonstrate organisms, cultures and guinea pig inoculation should be carried out. Cultures of the fluid, especially of the pellicle, on Petroff's medium is the method of choice.

The gram-positive cocci streptococci, pneumococci, and staphylococci grow readily in blood agar plates and beef heart infusion blood broth. In liquid media, the addition of 1 per cent dextrose or the substitution of 0.2 per cent sodium

phosphate for the sodium chloride content is sometimes used to enhance growth. The optimum P_H and temperature for incubation are 7.6 and $37^{\circ}C$, respectively. The organisms are differentiated by their characteristic growth in blood broth and blood agar and further by the examination of smears. The bile solubility test is frequently necessary to distinguish pneumococci from streptococci.

Of the gram-negative cocci the meningococcus is by far the most important and is found with some frequency while the gonococcus is recovered only rarely from the spinal fluid. They may be appropriately considered together as they both require a highly albuminous medium for growth. A blood agar plate containing from 30 to 50 per cent hydrocele or ascitic fluid is the medium of choice. It is necessary that the medium be freshly prepared and that the specimen be cultured as soon as possible as these organisms are markedly susceptible to cold or drying. Inoculation of the medium is best carried out by heavily streaking the plates with the spinal fluid and in addition pour plates may be made with this medium. The same precautions for possible loss of moisture should be observed as indicated in the section on blood cultures. Another useful method for culturing meningococci is to incubate the fluid for an hour or two before streaking plates.

Colonies of meningococci on clear mediums are transparent, lenticular in shape, and have a moist and smooth surface. Meningococci cannot be definitely differentiated from gonococci by manner of growth in culture mediums or by smear findings nor does the examination of their fermentative activities solve the question. The ultimate diagnosis of meningococci must, therefore, depend on the agglutination reactions. A polyvalent immune serum is generally used for this purpose but when a fine differentiation of meningococcal types is desired it is necessary to perform these tests with the three types of immune serum.

DISCUSSION

No attempt has been made to discuss every form of microorganism met with in blood or spinal fluid cultures, only those frequently found, or of practical importance are included.

With the realization that the tentative clinical diagnosis is frequently wrong, it is always advisable to perform routine examinations, as well as to carry out any special studies indicated.

Too much emphasis should not be placed on negative results. Many bacteremias have bacteria-free stages. In most cases this period is short, but in such diseases as subacute bacterial endocarditis it may cover a period of months.

The clinical interpretation of laboratory findings is sometimes very important. For instance streptococci have been isolated from the blood of patients with measles, chronic arthritis, rheumatic fever and even upper respiratory infections. To conclude that such patients have a streptococcal septicemia in the usual sense of the term would be erroneous. Such organisms are usually isolated by special technique, and are probably of low virulence, they may be present in small numbers and possibly at infrequent intervals. It is also conceivable that such organisms are present in the blood stream only in some ultra microscopic form later developing into the visible stage of their life cycle in

the blood culture medium. The question of secondary or terminal invaders also has to be considered.

In the average laboratory, unusual or unlooked-for organisms are generally discarded as contaminants. It is here recommended that such organisms be given careful consideration, as it is largely through study of the unexpected that our bacteriologic knowledge is increased.

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THE BACTERIOLOGY OF THE NOSE AND THROAT*

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IF WE consider the nose and throat as vestibules to the respiratory and alimentary tracts, and the relations of their vascular and lymphatic systems to the general circulation and the meninges, we are impressed by the avenues afforded to pathogenic bacteria for invasion and absorption of their toxic products. Further, a consideration of the diseases which may be spread in droplets of nasal and throat secretions emphasizes the importance of the clinical bacteriology of this region.

For convenience we shall discuss separately the flora of the nasal passages the accessory nasal sinuses the nasopharynx and the tonsils, but such a division is very inexact bacteriologically, because the continuity of the different parts the constrictions and tortuosities of the passages and the admixture of their secretions make it impossible to secure material from any one limited area uncontaminated by the bacteria from another.

Material for examination may be obtained by rubbing a sterile swab over the mucous membrane or by irrigating the passages with warm sterile salt solution Ringer's solution or broth. The use of a swab is a most convenient method and the only practical one when the secretion from a limited area is desired. To take material from the nasopharynx, a bent swab may be inserted through the mouth and behind the soft palate and rubbed over the nasopharyngeal walls. With proper care on insertion and withdrawal there is little danger of contamination from the mouth or tongue. In young children, or adults with exaggerated

*From the Bacteriological Laboratory of the Medical Division Metropolitan Life Insurance Company

gag reflexes, the West tube may help to minimize contamination of the swab with saliva, or a slightly bent swab may be passed through the middle meatus of the nose into the nasopharynx. Material taken in the latter way is necessarily contaminated by bacteria from the nasal passages. Irrigation of the nose and nasopharynx with a sterile solution may be employed when large amounts of secretion from the whole region rather than from one locality are desired. It is the method used by Olitsky and Gates¹ and other workers in studying the etiology of colds and influenza, and is especially useful when filter-passing forms are under investigation. Dochez² suggested sterile broth as the irrigating medium, because he believed that it preserved these bacteria and favored their filtration. Such material is frequently contaminated by bacteria from the mouth and nasal vestibule, and in comparison with nasopharyngeal swabbings contains fewer pneumococci and influenza bacilli, and more staphylococci of the albus variety, and diphtheroids.

Direct microscopic examination of the secretions is of limited value unless there is a demonstrable lesion with an exudate. Cultivation on suitable media will frequently show the presence of a suspected pathogen when direct microscopic examination fails to do so. Obviously the media employed must be adapted to the growth requirements of the particular organisms searched for, as for example, Loeffler's coagulated serum medium for the diphtheria bacillus, or hemoglobin media for the hemoglobinophilic group.

In discussing the bacteriology of any region an attempt is usually made to distinguish "normal" from "abnormal" flora. The reasons are obvious, but an exact differentiation is not possible because the flora is never constant, it varies in different persons, in the same person at different times, and in different geographical localities.^{3, 4, 5} Broadly speaking, certain species of bacteria occur more or less regularly in most healthy noses and throats, and may be considered "normal" flora. They are for the most part saprophytes and potential pathogens of low virulence. It is doubtful if virulent pathogens are ever normal flora, unless they be considered such in carriers in whom there are no demonstrable foci of infection. The "abnormal" flora consists of bacterial species occurring less constantly than those of the normal flora, it is transient except in persons with chronic lesions or foci of infection, and includes the more virulent pathogenic forms.

THE NOSE

The bacterial species of the healthy nasal mucosa have been listed by Bloomfield,⁶ Shibley, Hanger and Dochez,⁷ and Noble, Fisher and Brainard.⁴ Bloomfield considered that *Staphylococcus albus* and diphtheroids comprised the essential normal flora of the nose, and that gram-negative cocci, *B. lactis aerogenes* and hemolytic streptococci were normal transient forms. Dochez and his associates⁷ considered that staphylococci of the aureus and citreus varieties also belonged to the normal flora, and Noble, Fisher and Brainard⁴ found that green streptococci and pneumococci were occasional transients. Webster and Hughes,⁸ in studying the incidence and spread of pneumococci in healthy persons found these organisms more frequently in the nasal passages of children than in their

throats but in adults they found them less frequently in the nose. We may therefore conclude that the normal flora of the nose consists principally of diphtheroids and staphylococci of different varieties, that gram-negative cocci (*M. catarrhalis*, *M. crassus*, *M. flavus* and *M. siccus*) and pneumococci are common transients and that green and hemolytic streptococci occur but rarely as normal transients.

The abnormal flora of the nose is complex, and its clinical significance is by no means clear especially in the acute catarrhal diseases. Mackey⁹ reported finding *B. influenzae*, *B. pertussis*, and *B. mucosus capsulatus* in cases of chronic nasal catarrh in children. Bordet¹⁰ and Moncrieff and Lightwood¹¹ have found *B. pertussis* in the nasal mucus and mucopus of children with whooping cough. Meier and Steinert¹² isolated at autopsy the bacillus of Koch-Weeks from the purulent secretion of the nasal passages and from the spinal fluid of a fatal case of meningitis. Schulman¹³ and Hollender¹⁴ have reported nasal infections caused by Vincent's fusiform bacilli and spirochetes. Kistner¹⁵ found tubercle bacilli in primary tuberculosis of the nasal mucosa. Gonorrheal rhinitis with isolation of the gonococcus from the nasal pus was reported by Miller.¹⁶ Several varieties of gram-negative encapsulated bacilli (*B. mucosus capsulatus* group) have been found associated with rhinoscleroma and ozena. Jelin¹⁷ believed that they had no causative relationship because so many different fermentative varieties occurred in the same patient. Elbert and Guerless,¹⁸ however, considered one type, the bacillus of Frisch characterized by the formation of acid from glucose and saccharose only, to be of significance because of its presence within the infiltrations of granulomatous tissue together with the formation of specific immune bodies in the blood stream of patients. In cases of glanders, *B. mallei* is to be found in the nasal discharge by suitable methods of examination. The Klebs-Loeffler bacilli may be demonstrated in cultures from the nose in cases of nasal diphtheria.

THE ACCESSORY NASAL SINUSES

Because of the difficulty in securing uncontaminated material from the healthy sinuses in the living subject our most accurate information of their bacteria has come from studies made upon the sinuses of cadavers. Fraenkel¹⁹ examined 50 sinuses at autopsy, 28 were apparently healthy, and cultures from 13 of these showed no growth. In the remaining 22 sinuses (11 maxillary, 6 frontal, and 5 sphenoidal), pneumococci were the predominating bacteria found. Linton²⁰ studied normal sinuses in 26 cadavers within five to eighteen and one-half hours after death. After removal of the calvarium and brain the inner surface of the bone overlying the sinuses was sterilized by heat and trephined. Only the frontal sphenoidal and ethmoidal sinuses could be reached in this way, 74 per cent of the sinuses were sterile, the remaining 26 per cent yielded cultures of staphylococci, diphtheroids, *B. coli*, hemolytic streptococci, *M. catarrhalis*, and sarcinae. In examining a large series of maxillary sinuses in animals he found that 55 per cent in rabbits, 71 per cent in dogs, 83 per cent in guinea pigs, and 90 per cent in rats were sterile. It would therefore seem justifiable to conclude that the majority of healthy sinuses are bacteria free and that if bacteria gain entrance they are probably transients.

A number of different bacterial species has been found associated with inflammatory conditions of the sinuses. In 44 cases of acute sinusitis Babcock²¹ reported finding pure cultures of pneumococci in 24, staphylococci in 10, streptococci in 2, *M. catarrhalis* in 2, diphtheroids and *B. aerogenes* in 1 each, and no organisms in 4. In chronic cases the same author found staphylococci in pure culture 15 times, streptococci 4 times, pneumococci 3 times, and *B. mucosus capsulatus*, *M. tetragenous* and diphtheroids, once each. Ashley and Frick²² found in the maxillary sinuses of children *Staphylococcus aureus* in 23, *M. catarrhalis* in 18, *Staphylococcus albus* and green streptococci in 10 each, and *B. influenzae*, *B. pyocyaneus*, diphtheroids, and gram-negative encapsulated bacilli in one or two cases each. Eisner²³ in analyzing 50 operative cases of maxillary sinusitis reported that hemolytic and green streptococci predominated, but were more often found in nonsuppurative conditions. Tubercle bacilli^{24, 25} and Vincent's fusiform bacilli and spirochetes (anaerobic forms)²⁶ have been reported as causative agents in maxillary sinusitis. *B. mallei* has also been found in infections, especially of the maxillary and frontal sinuses.

THE NASOPHARYNX

The bacteria commonly found in the healthy nasopharynx have been reported upon by Jordan, Norton and Sharp,²⁷ Noble, Fisher and Brainard,⁴ Burkly and Smillie,⁷ and Milam and Smillie.⁸ Their results are based upon successive cultures taken from the same persons over long periods of time or upon single cultures from larger groups of subjects, the first method being of value in showing transient flora and the length of time it persists. There appears to be general agreement that green streptococci and gram-negative cocci are the predominating normal or basic flora of the healthy throat, that indifferent streptococci (Brown's "gamma" type),²⁸ pneumococci, staphylococci, nonhemolytic influenza bacilli, and diphtheroids are normal flora in some persons and frequent transients in others, and that hemolytic streptococci, *M. catarrhalis* and *B. mucosus capsulatus* are occasional transients. Burkly and Smillie,⁷ and Milam and Smillie⁸ found the normal basic nasopharyngeal flora in isolated communities in the subarctic, temperate and tropical zones to be, in general, the same with some minor differences. pneumococci were not found in normal throats in Alabama while they were quite prevalent in Labrador and the Virgin Islands, influenza bacilli occurred infrequently in Labrador, hemolytic staphylococci were very common in the Virgin Islands.

The variations in the nasopharyngeal flora in health make difficult a determination of the etiologic agents in acute catarrhal infections of the upper respiratory tract. There are already recorded in the literature countless attempts to determine the significance of certain organisms of this region in colds and in influenza. Pfeiffer's bacillus, discovered in 1892, was generally believed to be the cause of epidemic influenza until the pandemic of 1917-1918. During that outbreak Williams, Nevin, and Guile,³⁰ found it in 92 per cent of influenza cases and in only 40 per cent of healthy persons, but attempts to demonstrate a common epidemic strain resulted in showing such serologic heterogeneity as to cast doubt upon its etiologic significance. Mathers³¹ described a hemolytic

streptococcus found in the nose and pharynx of influenza patients during an outbreak in Chicago in 1915-1916. Attempts to implicate green streptococci have been made by numerous workers. In colds, changes in the normal flora have been noted by different investigators. Williams, Nevin, and Gurley³⁰ found that pneumococci, staphylococci and influenza bacilli were increased in number and frequency. Shibley, Hanger, and Dochez⁷ found that staphylococci of the aureus variety, hemolytic streptococci and influenza bacilli appeared late in colds, probably as secondary invaders. Noble, Fisher, and Brainard⁴ observed that pneumococci, staphylococci, indifferent streptococci and influenza bacilli were increased during colds. Burky and Smillie⁷ noted in Alabama the appearance of pneumococci of Group IV together with an increase of influenza bacilli when colds developed, while in Labrador an increase in the prevalence of influenza bacilli was associated with an epidemic of tracheitis.

Tunnichiff and Hovne,³² while studying measles, isolated a green diplococcus which at first grew only anaerobically but in later generations developed under aerobic conditions. They reported that serum from goats immunized to this organism seemed to afford some protection to persons who received it within three days of exposure.

The significance of certain organisms in the nasopharynx is well understood, as for example, the Klebs-Loeffler bacillus in diphtheria, and the hemolytic streptococci in scarlet fever and in septic sore throat. The presence of these virulent organisms in the throats of healthy persons would indicate that the latter are carriers. Meningococci may be found in the nasopharynx during the disease and also in carriers. *B. mallei* has been demonstrated in the pus from the nasopharynx in glanders and the *Spirocheta pallida* in the mucous patches of secondary syphilis. Tubercle bacilli have been found in tuberculous ulcerations of the nasopharyngeal mucosa.

THE TONSILS

The flora of the normal tonsil has been reported by Bloomfield⁶ and others to consist of gram-negative cocci, *Streptococcus viridans* and diphtheroids with *Staphylococcus albus*, *Staphylococcus aureus*, hemolytic streptococci and hemolytic influenza bacilli occurring as transients. Cobe³³ has found in addition pneumococci and *B. mucosus capsulatus*.

Many of these groups undoubtedly contain potential pathogens; the presence of virulent strains among them remaining undetected until an outbreak of disease. The Dicks have demonstrated that scarlet fever is produced by hemolytic streptococci of a specific type. Bloomfield and Feltz³⁴ found that certain persons consistently harbored hemolytic streptococci in their tonsils and others did not. Acute tonsillitis with the coincident appearance of hemolytic streptococci developed among the second group while the first remained healthy. They therefore believed that hemolytic streptococci were the inciting agents, and attributed the immunity of those in the first group to the fact that they were carriers. In a study of excised tonsils from 100 cases of acute tonsillitis, Polvogt and Crowe³⁵ found hemolytic streptococci the predominating organisms in 91 per cent. Pomaies³⁶ in his examination of 65 pairs of excised tonsils found

Staphylococcus aureus the predominating form in the majority of cases, with hemolytic streptococci ranking second, and considered both organisms to be of significance

The possible relationship to rheumatic fever of certain strains of streptococci of the throat has been suggested many times, it presupposes the existence of one or more specific types and their recovery with regularity from the throats, blood, and affected joints of rheumatic fever patients. These conditions have not yet been satisfactorily fulfilled. A mass of conflicting evidence has accumulated which is difficult to appraise. Andrews, Derrick, and Swift³⁷ found no evidences of serologic identity among their strains of hemolytic streptococci isolated from the tonsils, throats or hearts' blood of rheumatic fever patients. Small³⁸ and Birkhaug³⁹ have recently described strains of indifferent streptococci (gamma type) isolated from the throats, blood, urine, or feces in acute rheumatic fever and each has stated that his strains constituted a serologically homogeneous group. Nye and Seegal⁴⁰ were unable to confirm these observations, and Hitchcock⁴¹⁻⁴² has found the indifferent streptococci to be heterogeneous, and the different strains to occur with equal frequency in the throats of rheumatic fever patients and normal individuals. The work of Cecil, Nicholls, and Stainsby⁴³ is of especial interest, for they have isolated green streptococci in a high percentage of their cases from the blood and affected joints, and have proved the serologic identity of strains recovered from the two sources.

If the tonsils are involved in diphtheria, cultures from them will show diphtheria bacilli. Other bacteria which may produce diphtheria-like conditions have been reported. Gilbert and Stewart⁴⁴⁻⁴⁵ have recently described a pathogenic diphtheroid associated with outbreaks of sore throat, young cultures showed diphtheroid forms which became coccoid on longer incubation. Diphtheria antitoxin afforded little protection. Two cases of tuberculosis of the tonsils have been reported by Koplik⁴⁶ and Dickey⁴⁷. In both microscopic examinations of scrapings from the ulcers showed acid-fast bacilli. The case described by Dickey was probably a primary infection as a tuberculin test made one and one-half years before the appearance of the ulcer was negative, while one made afterwards was positive. Foster⁴⁸ reported acute tonsillitis in a patient who had contracted gonorrhea, a microscopic examination of smears from the tonsillar ulcer showed intra and extracellular gram-negative cocci, and organisms morphologically and culturally resembling the gonococcus were isolated. Busson⁴⁹ found *B. mucosus capsulatus* (Friedlaender's bacillus) in pure culture in a peritonsillar abscess. *B. mallei* may be found in the discharge from the nodules developing on the tonsils in glanders.

Diseases of the tonsils produced by fungi have been described by Castellani⁵⁰. In follicular tonsillomycosis and diphtheria-like tonsillomycosis the conditions were usually caused by yeast-like fungi of the genus *Monilia* but occasionally by other types. Hoffstadt⁵¹ from a white tonsillar membrane, isolated a saccaromyces which resembled that found by Kayser⁵² in cider. Smith⁵³ reported a case showing whitish patches on the tonsil, base of tongue, and lingual tonsil, an examination of the excised tonsil showed the presence of actinomyces. Rarer infections with the formation of granules in the tonsillar crypts have

been reported by Castellani⁵⁰. The granules contained masses of nocardia-like organisms leptothrix and vibriothrix, various bacteria and even protozoa such as spirochetes, amebae, and flagellates. Tunnichiff and Jackson⁵⁴ reported finding a vibriothrix in a tonsillar granule. Petzetakis⁵⁵ found amebae in the tonsils of two children with sore throats a few days before they developed amebic dysentery.

ANAEROBIC BACTERIA

The demonstration of anaerobic bacteria in the upper respiratory tract is complicated by the ability of most of the aerobic forms previously named to grow well under anaerobic conditions. A partial solution of this difficulty lies in filtering the respiratory secretions through Berkefeld "V" or "N" candles before planting them in culture media. Most of the aerobic bacteria and some of the anaerobic forms are removed by this procedure but many of the latter pass through with the filtrate.

Some anaerobic bacteria, however, have been isolated from unfiltered material from the nose or nasopharynx. Tunnichiff's⁵⁶ B rhinitis was found in the unfiltered nasal mucus of patients with acute coryza, she believed it to induce this condition when inoculated upon the nasal mucosa. Noble and Brainard⁵⁷ found *Staphylococcus parvulus* in unfiltered nasal washings. This organism, previously isolated by Veillon and Zuber⁵⁸ from an infected appendix and later by Lewkowicz⁵⁹ and others from the mouths of man and animals, is characterized by its ability to produce abundant gas in protein media and its inability to ferment sugars. Because of its prevalence it would seem to be part of the normal nasopharyngeal flora. Several strains of gram-positive cocci differing from one another in their fermentative reactions have been isolated from cases of rhinitis by Hall,⁶⁰ and Noble and Brainard⁵⁷. Gram-positive spirochetes have been found by Tunnichiff⁶¹ in cases of rhinitis and sinusitis. Gram-negative bacilli similar to those found in filtered washings, which are described below, have been reported by Noble and Brainard⁵⁷ in nasopharyngeal swabbings from normal persons and others with colds. Vincent's fusiform bacilli have already been mentioned in an earlier section.

The filter-passing anaerobic bacteria which have been described at one time or another are more numerous than those obtained directly from the unfiltered mucus. Olitsky and Gates⁶² isolated a gram-negative bacillus from filtered nasal washings of patients in the early stages of epidemic influenza and suggested it as the causative agent, they called it B pneumosintes because of its ability to injure the lung and predispose to secondary infections. When grown in Smith-Vogueli medium, it is a minute coccobacillus, on a solid medium such as coagulated blood agar, it is considerably larger. It produces acid from glucose, galactose, levulose, maltose, saccharose and lactose and specific agglutinins and other immune bodies in human beings and rabbits. Many other gram-negative bacilli have been found by different workers. Olitsky and Gates⁶² described three groups and two subgroups and Noble and Brainard⁶⁴ have differentiated twelve agglutinative and fermentative groups, these have been isolated from cases of epidemic influenza, rhinitis, or healthy subjects, and are probably part of the normal flora of the upper respiratory tract. Garrod⁶⁵ has reported a minute

gram-negative coccus occurring in hazy masses and Noble and Brainard⁶⁴ have found a similar form. It produces a characteristic colony on glucose serum agar but does not ferment the sugars. It is not pathogenic and is found in the majority of persons. Two species of gram-negative cocci have been described by Branham,⁶⁶ one of which appeared to be *Staphylococcus parvulus*, while the other differed from it in that it was hemolytic and formed no gas in protein media. Branham also found a minute gram-positive coccus. We have found small gram-positive bacilli, some resembling diphtheroids.

SUMMARY

From the foregoing paragraphs, it is evident that many bacterial species are to be found in the nasal passages and throat, and that the same, or apparently the same species occur on both the healthy and diseased mucous membrane. Therefore it is difficult, in the absence of a definite lesion, to appraise satisfactorily the clinical significance of many of the species encountered in a bacteriologic examination. This is especially true of the streptococci, whether green, indifferent or hemolytic, the pneumococci, staphylococci, gram-negative cocci and hemoglobinophilic bacilli. If we consider the hemolytic streptococci as an example, we must remember that they comprise a large group of many strains which differ greatly in their pathogenicity and virulence, and that while certain strains may give rise to scarlet fever or to septic sore throat, others may be innocuous. The finding of hemolytic streptococci, therefore, is not necessarily significant, but the finding of a particular strain may be of great significance. Unfortunately the demonstration of a particular pathogenic strain is not always easy and is usually impractical in routine examinations. The rôle of the anaerobic bacteria as incitants of colds and influenza is unknown, they would appear to be part of the normal flora and of little if any pathogenicity, but additional experimental evidence may change our views. The recent work of Dochez,⁶⁷ confirming the earlier work of Kruse⁶⁸ and Foster,^{69, 70} has again directed our attention to the probability that a filterable virus is the primary cause of certain respiratory infections, preparing the way for the secondary complications which may be brought about by many of the common bacteria harbored in the upper respiratory tract. We are probably correct in believing that in the presence of a definite lesion the finding of an organism of known or potential pathogenicity is clinically significant, that in the absence of a lesion it denotes a carrier but beyond this our present lack of knowledge does not justify definite interpretations.

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THE BACTERIOLOGY OF BILE

OBTAINED BY DUODENAL TUBE BILIARY DRAINAGE

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THIS article deals with the bacteriologic study of 1450 cultures made from the bile of 988 patients obtained during the course of duodenal tube drainage of the biliary system following the technic which we have advocated. That bile was allowed to flow from the patient through the duodenal tube into a culture flask containing Huntton's hormone broth, 20 to 30 drops of bile fluid being introduced into 100 c.c. of broth. In this sense therefore the title of this article is correct. But from another point of view all findings accredited to the bacteriology of the bile are justifiably debatable.

In the first place in taking cultures through a duodenal tube from the duodenum we are obviously culturing a zone into which is being poured secretions from sources other than the biliary system, namely, the secretions of the respiratory tract of the mouth (notably the saliva), of the stomach, the pancreas and the duodenum itself. Viable bacteria from any of these sources ultimately reach the duodenum if they can successfully pass such bactericidal barriers as our bodies possess. Obviously this would make inferences as to the primary source of positive cultures from the duodenal zone somewhat doubtful. Scientifically speaking this is the sounder view to take and this position has emphatically been chosen by some investigators of this problem. However I believe and hope to show that in many patients despite certain obvious inaccuracies bacteriologic inferences can be made which are of such value both in diagnosis and in treatment as to make it imperative that we do not entirely disregard this aid in diagnosis.

In medicine, as in other branches of science the ultimate truth is long delayed. The opinions expressed in one decade or one medical generation or in one century are often fully accepted for the time being and often totally disregarded by the next generation because methods have been improved for reappraising former views.

Thirty years or more ago it was generally accepted that the *fasting duodenum in health* was amicrobic, whereas bacteria ingested with the food and contaminated by bacteria resident in the mouth and upper and lower respiratory tracts, resistant to the so-called bactericidal power of the acid gastric juice could be readily cultivated from the duodenum when operated upon. Such cultures chiefly yielded yeast and various cocci.¹ Twenty years ago after the duodenal tube had been perfected other investigators^{2, 3} showed that viable bacteria could be readily cultivated from the fasting duodenum in health and also with particular frequency in gastrointestinal disease. However the view was held that such findings were inaccurate because of contamination by

bacteria carried from the mouth, esophagus, and stomach by the duodenal tube itself. Thirteen years ago, after the advent of nonsurgical drainage of the gall tract,⁴ we added routine cultures from the duodenal zone and became enthusiastic in our belief that, with certain precautionary attempts to sterilize the mouth and stomach, cultures could be secured which would furnish inferential evidence of the source from which such bacteria were obtained. Following my earlier publications⁵ renewed interest was indicated in various papers.⁶⁻²⁰ The opinions of some of these authors supported, while others were unable to confirm the views which I had expressed, chiefly because of the obvious factors of contamination. No one can dispute or disregard the existence of such factors. It is necessary, however, to emphasize that with care in technique such factors can be at least partially controlled.^{5b}

In health the mouth, its contents, and the accessory cavities which empty into it (the respiratory passages, sinuses, and ears) constantly contain various bacteria, many of which are pathogenic, but the normal resistance of the tissues and the antibacterial defenses (lymph nodes, etc.) minimize their effect. In disease (bronchitis, nasopharyngitis, sinusitis, otitis media, eustachian-tube infection, gingivitis, pyorrhea, dental caries and root infection, tonsillitis, salivary duct infection) the mouth cavity is a most potent source of infection through lymphatic and blood distribution and by direct bacterial descent (swallowed saliva) in the production of subsequent gastrointestinal disease in its acute and chronic forms (gastroduodenitis, peptic ulcer, cholecystitis, hepatocholangitis, emphysema, enteritis, appendicitis, colitis). In the preceding groups the pyogenic cocci are particularly common. One of the greatest factors influencing the causation of such secondary disease is the individual tissue susceptibility and the tissue resistance to such infection. A second factor of less importance is the bactericidal power of the acid gastric juice and its various components.²¹

Every gastroenterologist is at times amazed to see the frequency of extensive oral sepsis, especially in patients from rural communities, which exists in certain patients without evident acute or chronic gastrointestinal disease or of infectious arthritis, endocarditis, neuritis, and the like. No doubt, many such persons acquire slowly developing immunity to the bacteria harbored in such dirty mouths. On the other hand, even in health various air-borne bacteria, such as influenza, may overwhelm the person and cause acute intestinal disease, likewise various food, water, or insect-borne bacteria may produce acute endemic or epidemic enteric disease by overwhelming the nonimmune host, as occurs in typhoid, paratyphoid, and various other bacillary and coccus infections. It would seem, therefore, that much depends on the virulency of the bacterium measured in terms of tissue resistance and in the selective affinity of certain tissues to certain strains of virulent bacteria, as pointed out by Rosenow.

The 988 cases on which this study is based occurred in my private practice between the years of 1920 and 1926. These patients were going through the usual routine diagnostic appraisal for gastrointestinal disease or its absence. All of them had one or more cultures made of bile discharged into the duodenum and recovered by the duodenal tube. Of these 988 patients 554 were males and 434 were females. Classified by half decades from youth to old age, Table I indicates that over two thirds of the patients were between the ages of thirty and

fifty-four years and the largest number of patients for any single decade was between thirty-five and forty-four years

Of the total number of patients, 64 furnished no evidence of gastrointestinal disease after complete study and represent the average normal adult. This group furnished the highest percentage of sterile cultures. A second group of 93 patients on final appraisal represented cases of various functional gastrointestinal disorders. This group furnished the next highest percentage of sterile cultures. A third group of 162 cases on final appraisal represented various grades of organic gastrointestinal disease, but without recognizable clinical or laboratory involvement of the biliary system. Of these the 32 who came to the operating table showed no evidence of gall bladder disease. A fourth group of 404 patients were cases of biliary tract disease, easily recognized by history, physical examination, roentgen ray examination, laboratory studies, including biliary drainage, and in 101 instances were confirmed by primary gall bladder surgery. I consider it significant that in these 101 patients the culture obtained at the operating table from the gall bladder was identical with that recorded in the preoperative drainage culture in a definite majority of cases. A fifth group of 69 cases represented patients who had already undergone one or more operations on the biliary system (cholecystostomy, cholecystectomy, cholecystoduodenostomy, release of adhesions, etc.) and who suffered continued morbidity. A sixth group of 154 patients presented vague atypical symptoms that were not characteristic of

TABLE I
988 PATIENTS ANALYZED BY AGE AND SEX

Years	10 to 14	15 to 19	20 to 24	25 to 29	30 to 34	35 to 39	40 to 44	45 to 49	50 to 54	55 to 59	60 to 64	65 to 69	70 to 74	75 to 79	Total
Males	3	6	22	46	76	86	88	68	66	36	29	24	2	2	554
Females	3	4	22	49	54	83	66	44	54	24	12	17	2	0	434
Total	6	10	44	95	130	169	154	112	120	60	41	41	4	2	988

any of the usual gastrointestinal or biliary tract syndromes, and who would otherwise have remained unrecognized as biliary tract suspects or Grades I or II biliary tract disease, were it not for the evidence of abnormal cytology and positive cultures of pathogenic bacteria obtained by biliary tract drainage. This group, most important to recognize at an early stage of their disease, yielded 15 per cent of the positive cultures. This group also yielded the largest number of successful results from therapeutic biliary drainage by duodenal tube. A seventh group of 74 patients were cases which clearly overlapped, chiefly with Groups 4 and 5, and represented various types of liver disease (cirrhosis, hepatitis, cholangitis, cancer).

The histories of this series of 988 cases showed that only 333 patients had not been operated upon for any condition. Of the 988 patients 316 had already undergone 434 abdominal operations. In many instances two or more abdominal organs had been surgically treated at the one operation, so the following figures overlap. 269 patients had already had their appendices removed, 69 patients had previously undergone gall bladder surgery, of which 34 were cholecystos-

tomies, 32 cholecystectomies, and 3 cholecystoduodenostomies. Of this group I had to refer 14 patients for further gall tract surgery. The remainder of this group of 316 cases were made up of pelvic surgery, gastrojejunostomy or other operations for peptic ulcer, colostomy, release of adhesions, and hernial repairs. Of this group having had abdominal surgery 228 had been operated on once, 63 patients twice, 20 patients three times, and 5 patients four times. From the foregoing it will be noted that I had to refer 147 patients for abdominal surgery—101 for primary gall bladder operations, 14 for reoperation on the gall tract, and 32 for surgery other than gall tract.

Although this data may appear irrelevant to the subject of this paper I feel that it is necessary to tabulate and classify the general character of the material studied by duodenobiliary culture, because it serves better to account for bacteriologic discrepancies between this series and others that might be studied in a more general practice.

In addition, there were among the 988 cases 339 patients who had been operated for various conditions requiring nonabdominal surgery (tonsils, adenoids, turbinates, sinuses, hemorrhoids, pelvic plastic repairs, etc.). Of this group 210 patients had been operated upon once, 93 patients twice, 31 patients three times, 10 patients four times, and 4 patients five times. Many of these patients had also undergone one or more abdominal operations. For instance, one patient had had four abdominal operations and five otherwise. Two hundred and seventy-four patients had already had their tonsils removed, in whom this source of contaminative infection in the cultures reported was eliminated, and I recommended tonsillectomies in 156 patients who had evident tonsillar focal infection.

Although, in the preparation of this paper insufficient time was available to classify the number of patients who had gingivitis, pyorrhea, dental caries or root infection, previous histories of or present symptoms of sinusitis, a cursory study of the case records suggested at least 25 per cent of such present or antecedent involvement. It is evident then how important such focal infection above the level of the stomach can be in the etiology of gastrointestinal disease.

TABLE II
SUMMARY OF BILE CULTURES

	PATIENTS	CULTURES	STERILE	CONTAMINATED	PURE	MIXED
Total No	988	1450	240	188	629	393
Per cent			16.5	12.9	43.4	27.1

An analysis of antecedent or present skin jaundice in the total group of 988 patients was significant in that of 274 patients who gave such histories, 61.8 per cent yielded positive cultures from the duodenobiliary fluid. It was interesting to me that of a total group of 184 patients who gave histories of an antecedent typhoid fever, only 3 patients yielded bile cultures of *B. typhosus*.*

*One of these cases was reported on page 522 of the writer's monograph (see Ref 5-K) a second one of a typhoid carrier of twenty-six years duration finally cured, will shortly appear in the *Journal of the American Medical Association*.

Table II indicates that on the 988 patients a total of 1 450 cultures of the duodenobiliary fluid were made. Of these, 240 or 16.5 per cent yielded sterile cultures, 629 or 43.4 per cent yielded pure cultures of one bacterium only, 393, or 27.1 per cent yielded mixed cultures of two or more bacteria, 188, or 12.9 per cent, yielded contaminated cultures.

We considered the cultures contaminated when they yielded *B. subtilis*, yeast, *Leptothrix mivehia* or other moulds.

B. subtilis occurred 28 times or 14.9 per cent of the contaminated group but only 1.9 per cent of the total number of 1,450 cultures, yeast occurred 16 times or 8.4 per cent of the contaminated group but only 1.1 per cent of the total number of 1,450 cultures, moulds occurred 12 times or 6.4 per cent of the contaminated group but only 0.8 per cent of the total number of 1,450 cultures. We also considered as contaminants *Streptococcus salivarius*, *Staphylococcus albus*, the diphtheroids, *Micrococcus catarrhalis* and the pneumococcus.

Streptococcus salivarius occurred 19 times or 10.1 per cent of the contaminated group but only 1.3 per cent of the total number of 1,450 cultures, *Staphylococcus albus* occurred 71 times or 37.7 per cent of the contaminated group but only 4.9 per cent of the total number of 1,450 cultures, diphtheroids occurred 15 times or 7.9 per cent of the contaminated group but only 1.03 per cent of the total number of 1,450 cultures, *Micrococcus catarrhalis* occurred 16 times or 8.4 per cent of the contaminated group but only 1.1 per cent of the total number of 1,450 cultures, pneumococcus occurred 11 times or 5.8 per cent of the contaminated group but only 0.8 per cent of the total number of 1,450 cultures. In many instances with *B. subtilis*, yeast or moulds freshly examined spreads of bile stained floccules indicated that other bacillary or coccoid groups were present but failed to grow out in differential subcultures.

Table III indicates that in the pure cultures, in which only one bacterium was isolated, the three main groups were the streptococci 25 per cent, the *Staphylococcus aureus*, 34.6 per cent, and the *B. coli* group, 39.3 per cent which together represent 98.9 per cent of the total. The remainder are divided between *B. pyocyaneus*, *B. lactis aerogenes*, *B. typhosus*.

TABLE III
SUMMARY OF PURE CULTURES

CASES	STREPTOCOCCUS				STAPH. AUREUS	B. COLI	B. PYOCYAN.	B. LACTIS AEROG.	B. TYPHOSUS
	HEMOL.	NON-HEMOL.	VIRID.	TOTAL					
Total No (629)	93	59	5	157	218	247	3	1	3
Per cent	14.8	9.4	0.8	25	34.6	39.3	0.47	0.15	0.47

Of the 393 mixed cultures containing two or more bacteria the four main groups are *B. coli* and *Staphylococcus aureus*, *B. coli* and nonhemolytic streptococcus, *B. coli* and *Staphylococcus albus*, nonhemolytic streptococcus and *Staphylococcus aureus*. In these groups *B. coli* occurred 194 times. If these were added to the 39.4 per cent of pure *B. coli* cultures, *B. coli* would dominate

the incidence of all other bacteria. This may be important in estimating the influence of *B. coli* in contributing to the production of the symptoms of hepatic intestinal toxemia. It might be even higher than this percentage if the formula of Hüntoon's hormone broth were not titered to retard the growth of *B. coli*.

While giving due consideration to the fact that the pyogenic cocci (streptococci 25 per cent and *Staphylococcus aureus* 34.6 per cent) which occurred in 59.6 per cent of all pure cultures, might also represent contaminations from above the duodenal zone in many instances, perhaps even in a majority, nevertheless an analysis of Groups 3, 4, 5, and 7 will indicate that in many patients these bacteria also invade the biliary tract and produce localized infection which can best be detected, prior to operation, by cultures of the duodenobiliary fluid obtained by the duodenal tube.

Group 3 (Table IV) consisted of 162 cases of organic gastrointestinal disease (chiefly gastroduodenitis, stomach or duodenal ulcers, upper right quadrant adhesions, appendicitis, colitis), but without recognizable biliary tract disease. Of these, 42 were confirmed as such by x-ray study but without operation, and 32 were proved at the operating table. In this group, 39 patients (24.1 per cent of the group) yielded sterile cultures, 24 patients (14.8 per cent of the group) yielded contaminated cultures, and 99 patients (61.1 per cent of the group) yielded positive cultures.

TABLE IV

GROUP 3 ORGANIC GASTROINTESTINAL DISEASE BUT WITHOUT BILIARY TRACT DISEASE

CASES	CULTURES			DIAGNOSIS CONFIRMED BY X RAYS BUT WITHOUT OPERATION	DIAGNOSIS PROVED AT OPERATION
	STERILE	CONTAMINATED	POSITIVE		
Total No (162)	39	24	99	42	32
Per cent	24.1	14.8	61.1		

Group 4 (Table V) consisted of 404 cases easily recognizable as gall bladder or duct disease. A number of them overlapped Group 7 with liver disease, and a number with Group 3 because in some instances there were both cholecystitis and peptic ulcer or upper right quadrant adhesions. The bacteriologic findings, however, have been listed but once. Of these 404 cases 108 were confirmed by x-ray study, but without operation, and 101 were proved at the operating table.

TABLE V

GROUP 4 GALL BLADDER OR DUCT DISEASE

CASES	CULTURES			DIAGNOSIS CONFIRMED BY X RAYS BUT WITHOUT OPERATION	DIAGNOSIS PROVED AT OPERATION
	STERILE	CONTAMINATED	POSITIVE		
Total No (404)	37	39	328	108	101
Per cent	9.2	9.7	81.1		

In this group 37 patients (9.2 per cent of the group) yielded sterile cultures, 39 patients (9.7 per cent of the group) yielded contaminated cultures and 328 patients (81.1 per cent of the group) yielded positive cultures.

Group 5 (Table VI) consisted of 69 patients who showed continued morbidity after having undergone one or more operations on the gall bladder. Some of these patients also overlapped Group 7, having liver disease. In this group

TABLE VI

GROUP 5 CONTINUED MORBIDITY AFTER ONE OR MORE OPERATIONS ON GALL BLADDER

CASES	CULTURES		
	STERILE	CONTAMINATED	POSITIVE
Total No (69)	7	8	54
Per cent	10	11.6	78.4

7 patients (10 per cent of the group) yielded sterile cultures, 8 patients (11.6 per cent of the group) yielded contaminated cultures, and 54 patients (78.4 per cent of the group) yielded positive cultures.

Group 7 (Table VII) consisted of 74 patients with liver or duct disease (hepatitis, cholangitis, cirrhosis, cancer—2 cases). In this group 5 patients (6.8 per cent of the group) yielded sterile cultures, 6 patients (8.1 per cent of the group) yielded contaminated cultures, and 63 patients (85.1 per cent of the group) yielded positive cultures. It may be of interest that of the positive cultures *B. coli* was isolated 24 times, *B. typhosus* in 3 instances, *B. pyocyaneus* once, and the pyogenic cocci 46 times.

TABLE VII

GROUP 7 LIVER OR DUCT DISEASE

CASES	CULTURES		
	STERILE	CONTAMINATED	POSITIVE
Total No (74)	5	6	63
Per cent	6.8	8.1	85.1

Comparing the analysis of these four groups, it becomes apparent that there is a distinctly higher percentage of positive cultures in Groups 4, 5, and 7 (patients with biliary tract disease) than in Group 3 (patients without biliary tract disease). Furthermore, both the sterile and contaminated groups in biliary tract disease are smaller than occurs in gastrointestinal disease, despite the fact that the same contaminating organisms, if contaminants, reach the duodenum in both groups.

I have already suggested that the interested reader of this article should carefully study the chapter in my monograph dealing with the bacteriologic

methods," both from their clinical discussion and from the technical routine worked out by John A. Kolmer, because of the fact that so much greater detail is therein described, which obviously is not appropriate to this paper. In that chapter certain tables will indicate that Kolmer found that although various strains of streptococci and staphylococci were recovered in a large percentage of cases, nevertheless there was also a high incidence of the recovery of *B. coli* groups. Furthermore, of these *B. coli* groups several strains were definitely pathogenic to inoculated animals. On the death and autopsy of such animals the *B. coli* was recovered with impressive frequency from the portal blood and from the gall bladder bile, and furthermore, in a number of instances a gram-negative rod was demonstrated in the tissues of the gall bladder wall. Similar findings were also noted many times in rabbits inoculated with the progenic cocci. This may be significant.

However in this paper it might be well to emphasize that the doctor or carefully trained technician should be on the alert, when taking cultures from the bile, to select through the glass observation window of the tube bile stained rather than imbile stained floccules. The former are more apt to represent bacterial invasion of the biliary system, the latter, more apt to represent contamination brought down from the upper respiratory passages, mouth, or stomach. Also in the selection of such bile-stained floccules additional care should be excited not to select the large shaggy or slimy bile-stained floccules which under fresh spread appraisal indicate the characteristic microscopic picture of oleaginous degeneration so common to cystic duct catarrh and possibly cholesterosis.²² The microscopic examination of such spreads is rarely as rich in bile-stained bacterial colonies as are the small fine, feathery or granular bile-stained floccules, often as minute as pinpoint size. Under microscopic appraisal such floccules more frequently reveal densely bile-stained colonies, in association with bile-stained pus cells and greater degrees of exfoliation of tall or short bile-stained columnar epithelium, in the characteristic fan-shaped or rosette clusters, which are supportive evidence of inflammatory disease of the mucosa of the gall bladder or ducts.

For some time I have been impressed with the frequency with which the *B. coli* groups have been recovered in bile drainages from patients who exhibit symptoms of what I have described as hepatic intestinal toxemia.²³ This would appear to suggest an impairment or loss of power of the bacterial function of the liver. This function seems to be a definite one and much of the published literature concerning it is already well known.²⁴

Even in health there is evidence of a constant transfer of *B. coli*, the most common bacterial group in the human intestine, into the mesenteric veins, thence to the portal vein, and thence to the liver cells. These cells in health appear to have the power of destroying completely the germs brought to the liver (a bacteriolytic power) or of killing them and thus rendering them harmless for redistribution (a bactericidal power). Furthermore, there is general agreement that the *B. coli* group is chiefly resident in the ascending colon, in the cecum, and in the terminal loops of the ileum, and they become progressively less in

*See Reference 5-K chapter 19 pp 347-366

numbers as one ascends the ileum to the duodenum. In other words in health we are not accustomed to see *B. coli* invasion of the duodenum or the stomach, although obviously in a minority of cases such cultures can be obtained, but the explanation is not far to seek.

Therefore, in connection with our studies on the microscopy and culture of bile stained floccules obtained on biliary drainage we have frequently noted the two following points. That in taking cultures from bile which has yielded *B. coli* one group requires from eighteen to twenty-four hours for its demonstration in culture flasks whereas in another group of patients an extraordinarily luxuriant growth of *B. coli* has occurred within two or three hours following the planting of the culture. This latter group therefore leads us to the suspicion that in such a patient both the bacteriolytic and bactericidal power in the liver cell is reduced or destroyed. The second point has been our observation that despite the recognition in our fresh spreads of large numbers of *bile-stained* bacterial colonies, morphologically approximating the appearance of *B. coli* groups such bacteria have failed to be cultivable and have yielded us sterile cultures. In other words such bacteria do not appear to be viable. This leads to a surmise that in such cases the bactericidal activity of the liver is still preserved but its larger function of complete bacteriolysis has been reduced or destroyed.

It seemed desirable, therefore to analyze an eighth group (Table VIII) consisting of patients who exhibited symptoms of hepatic intestinal toxemia in the attempt to learn whether the *B. coli* group would appear conspicuously frequent. This turned out to be a large group of 385 patients and necessarily overlapped Groups 3 to 7. *B. coli* was recovered in pure culture in 56.7 per cent, and *B. coli* in mixed culture together with streptococci or *Staphylococcus aureus*, *B. pyocyaneus*, *B. lactis aerogenes* and various enterococci was recovered in 23.1 per cent. Thus *B. coli* was recovered in 79.8 per cent of the entire group.

TABLE VIII
GROUP 8 HEPATIC INTESTINAL TOXEMIA

CASES	CULTURES				
	STERILE	CONTAMINATED	B. COLI PURE	B. COLI MIXED	PURE OR MIXED WITHOUT B. COLI
Total No (385)	24	25	218	89	29
Per cent	6.2	6.5	56.7	23.1	7.5

The bacteria mentioned above, either in pure culture or mixed groups (but without *B. coli*), occurred in 7.5 per cent, contaminating cultures occurred in 6.5 per cent, and sterile cultures occurred in 6.2 per cent of this group.

To me this analysis appears to have significance because here the element of contamination from above the duodenal zone is reduced to its minimum because the *B. coli* group can be cultivated from the duodenal zone in health with relative infrequency and because such a high recovery of 79.8 per cent of *B. coli* in patients exhibiting symptoms of hepatic intestinal toxemia argues in favor

of a breakdown in the bactericidal function of the liver as an etiologic factor of importance

As a result of the foregoing analyses I retain my conviction, despite the evident inaccuracies to which I and other investigators have called attention, that with careful technique in duodenobiliary drainage in regard to its bacteriologic aspects such a large number of positive cultures, as above defined, are obtained in biliary tract disease as to make it a matter of regret were we totally to disregard its diagnostic value and its therapeutic application

Aside from the recognition of typhoid carriers of the hepatocholangitis type, and its usefulness in the detection of a *B. pyocyaneus* hepatitis in a case already reported twice,* its usefulness in the following case, selected from many similar ones in Group 7 of coccus infections of the liver and ducts, would alone justify its continued usage

CASE No 1920—G H D, suffering from recurring attacks of jaundice with chills, fever, and sweats, was forty nine years old when I first saw him in April, 1926. His past history was of importance. Although a healthy country bred boy, with usual outdoor activities, as far back as he can remember he had bilious attacks, accompanied by headache, nausea, and vomiting. At twenty he had an attack of severe pain in the right hypochondrium not relieved by the usual measures, and accompanied by nausea. At twenty three, because of supraorbital headache, blurred vision, and sleepiness after reading, his eyes were examined and compound hyperopic astigmatism was discovered and corrected with relief of these symptoms. His eye grounds were negative. At thirty eight he developed muscae volitantes which could not be seen with the ophthalmoscope. About this time he noticed tenderness in finger joints and recurrence of sciatica and lumbago, attacks of which he had suffered in childhood. He noticed a deep yellow stain appearing on his underclothes and pajamas under the armpits due to sweating. He was then mildly jaundiced. Over the next three years, upper abdominal symptoms of indigestion became more pronounced, and his weight gradually decreased from 190 to 160 pounds and the muscae volitantes became very troublesome. In 1922, at the age of forty five, his leucocyte count was 13,000, therefore, his tonsils, which contained pus and streptococci, were removed. Four days later he had a severe attack of typical gall stone colic. X ray examination showed considerable osteo arthritis of the lumbosacral region, causing limited lateral rotation of the spine, but because the x rays did not reveal stones, operation was deferred until October, 1923. His gall bladder was found to be small and thickened, and to contain 25 mulberry stones ranging from sand to pea size and one stone of olive size. The gall bladder and a diseased appendix were removed. The upper abdominal incision broke down and drained bile copiously although the cystic duct had been securely ligated. During this time he had severe nocturnal pains in the right chest and particularly under the right shoulder blade simulating severe pleurisy, together with drenching night sweats. The feces became clay colored, the urine dark, and he became temporarily jaundiced. The abdominal sinus drained until December when it superficially closed for five days. On the fifth day headache, general aching, severe chills, and fever ensued and the region of the wound became tender. It was reopened and two ounces of blood stained bile was aspirated and cultured, yielding streptococci. The wound drained for three days and finally closed. Four months later headache, malaise, and tenderness over the upper right quadrant were followed by chills and fever (104° F) for forty eight hours which terminated after a profuse sweat. Clay stools and jaundice lasted for a week. This was the first of a series of similar attacks that occurred at frequent intervals. His stools were cultured and yielded a streptococcus. A vaccine was given but failed to influence the attacks. He then had his teeth and gums treated for pyorrhea and one infected molar was extracted. It is significant that on one occasion within twenty four hours after removing tartar and cleaning the

*See Reference 5-K, page 501 and Reference 22, page 806

teeth, he developed what was considered catarrhal jaundice, and a second attack within two days after extracting the infected molar tooth. Shortly after this time he came under my care.

His general appearance placed him in the liver group. His sclerae were markedly jaundiced, his skin was lemon yellow, his liver was considerably enlarged, and his spleen slightly so. The van den Bergh test gave a delayed direct reaction and the quantitative bilirubin of the blood was six times the normal. The leucocyte count was 9,200. He was placed in the hemolytic jaundice group. Biliary drainage gave evidence of hepatocolangitis with a suspicion of a residual stone. Culture yielded hemolytic streptococci only. His tonsils had already been removed, and cultures from his throat, his teeth, and gums failed to produce streptococci, so by inference his previous vaccine course might have whipped this focus without affecting his liver infection.

After it became evident that biliary therapeutic drainage with vaccines was not controlling his attacks, and the evidence of residual stone became more definite, he was referred for operation in April, 1927. The common duct was found partially strictured, behind which a small stone was found and removed. The stricture was dilated and T tube drainage instituted. Cultures at the operating table and subsequently from the bile recovered by T tube drainage yielded hemolytic streptococci in pure culture. T tube drainage was continued for about seven weeks and on its removal drainage ceased, the wound healed, the sinus closed, and the patient has had no recurrence of chills, fever, or sweats accompanied by jaundice, although he has had three attacks (reported by telephone "follow up" in January, 1932) of chills followed in twenty-four hours by "gouty" manifestations of the great toe.

After analyzing all of the data as presented in this paper we can see that there should be no great objection to considering that the *B. coli*, *B. typhosus*, and *B. proteus* are not contaminants but genuine infections. What argument exists, therefore, is concerned with the group of pyogenic cocci. In my opinion they should be considered in many cases to be transplants rather than contaminations as illustrated in the foregoing case.

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LABORATORY METHODS IN THE TREATMENT OF PNEUMONIA*

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PNEUMONIA from a bacteriologic and immunologic viewpoint has been a development of the last fifty years. It was not until 1880 that Pasteur and Steinberg independently described the pneumococcus, and two years later that Koch recognized the tubercle bacillus. Frankel, in 1884, suggested that the pneumococcus was an important etiologic agent of pneumonia, and Weichselbaum, shortly after, provided conclusive evidence of this fact by obtaining pneumococci from a number of postmortem cultures. At about the same time that the pneumococcus was described, other bacteria, such as the staphylococcus, streptococcus, and Friedlander's bacillus were also being studied, and their morphology becoming known. These epochal discoveries were accompanied by the accumulation of important information regarding staining methods, culture media, animal pathogenicity, et cetera. In 1910, Neufeldt and Handel discovered that although the various strains of pneumococci were similar in morphology, cultural characteristics, sugar reactions, and bile solubility they were immunologically different. These investigators laid the foundation for a biologic classification of the pneumococci which was constructed by Dochez and Gillespie when they separated the pneumococci into Types I, II, and III, and Group IV.

Since the recognition of the immunologic types, the most important progress that has been made has been the development of specific treatment. However, there has been a great deal accomplished also in the bacteriologic and chemical studies of the pneumococcus. The organism itself has been divided into its various chemical fractions. The toxic and immune substances produced by these bacteria have been the subject of numerous investigations. The most recent noteworthy advances have been in the improvement of methods already known, of these, modifications of sputum typing and blood culture methods which give more rapid and more accurate results seem to have the greatest practical significance.

Upon the knowledge gained through these modern methods of bacteriology and immunology, a sound etiologic diagnosis of pneumonia has been established which changes our whole conception of this disease. The mere designation of

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pneumonia as lobar broncho, or lobular, has great limitations in its aid to treatment prognosis, and even to a general understanding of the disease

The importance of distinguishing between a case of pneumococcus pneumonia and one of tuberculous pneumonia is generally recognized, today. Nevertheless such a necessary differentiation cannot always be made by purely clinical means, but usually can be definitely made with the aid of laboratory methods. Neither can such diseases as hemolytic streptococcus pneumonia and Friedlander's bacillus pneumonia be differentiated by clinical methods and here, again, laboratory procedures must be employed. Still the prognosis and attempts at specific treatment in these diseases are entirely dependent upon this etiologic diagnosis.

A complete bacteriologic study of each case of pneumonia including examination and typing of the sputum, culturing of the blood and, when indicated, culturing of the spinal and chest fluids, is indispensable for a thorough understanding of that case. At Bellevue Hospital, since 1920, a number of routine bacteriologic procedures have been carried out on all cases of pulmonary disease as part of an investigative study, but more and more have we become convinced of the practical significance of many of these procedures. At the present time our routine is as follows:

- 1 As soon after admission as the diagnosis of pulmonary disease is made, a sputum specimen is obtained. If the patient is not expectorating, a throat swab is procured. The specimens obtained from patients with pneumonia or suspected of pneumonia, are sent to the laboratory for examination and typing. Sputum procured from patients with chronic pulmonary disease is examined and studied microscopically by the Ziehl-Neelsen and dark-field methods.

- 2 At the same time that the sputum or swab is obtained, cultures are made from the blood of all patients with pneumonia, or suspected pneumonia.

- 3 When the first sputum obtained from pneumonia patients has not shown a predominance of Type I or Type II pneumococcus, a second specimen is procured in order to confirm the initial findings. If sputum specimens of tuberculous patients give negative findings, other specimens are obtained, at regular intervals, for microscopic study.

- 4 When initial blood cultures taken on pneumonia patients are positive, they are repeated daily until the procedure is no longer indicated, when negative, they are repeated every second day until the toxemia subsides.

- 5 Spinal fluids, chest fluids, joint fluids and pus obtained from infected foci are obtained whenever possible and sent to the laboratory for bacteriologic study.

- 6 Exudate is occasionally obtained by lung puncture and then only when the sputum or swab gives ambiguous or unsatisfactory results.

DIAGNOSIS OF PNEUMONIA

It has already been pointed out that the real value of the various laboratory procedures lies in the fact that they supply the information upon which depends the diagnosis not only of the condition itself, but also of its complications. In other words, the etiologic diagnosis is based upon the laboratory findings just as, in turn, the treatment is based upon the diagnosis.

Pneumonia, like many other diseases, has been classified according to a number of different systems, the two most important being the anatomic and the etiologic. In the anatomic classification, pneumonia is divided into lobar, broncho, and lobular pneumonia. This is still the most commonly accepted clinical classification, although it is not nearly as exact, and certainly not as significant, as the etiologic one. Cole has suggested the division of pneumonia into acute lobar pneumonia, primary atypical pneumonia, and secondary atypical pneumonia, determined primarily by the character of the onset and the course of the disease. The value of the anatomic classification and that of Cole is that through clinical findings they approximate the true etiologic diagnosis.

The etiologic classification of pneumonia is as follows:

- 1 *Pneumococcus pneumonia*
 - A Type I
 - B Type II
 - C Type III
 - D Group IV
- 2 Hemolytic streptococcus pneumonia
- 3 *Staphylococcus pneumonia*
- 4 Friedlander's bacillus pneumonia
- 5 *Bacillus influenza pneumonia*
- 6 Mixed infections
- 7 Infections of unknown etiology

Clinically, it is impossible to differentiate the various etiologic varieties of pneumonia. Most of the pneumococcus pneumonias cause a lobar consolidation and run a typical course, but any one of the other kinds may follow the same course and show the same physical findings. On the other hand, the miscellaneous varieties (hemolytic streptococcus, Friedlander's bacillus, etc.) usually run an atypical course and have the signs of bronchopneumonia but they may vary also. Furthermore, there is a great deal of overlapping of the lobar, broncho and lobular forms, and, likewise, it is not always easy to decide whether the course is typical or atypical. The etiologic classification, which is based on the infecting organism, is the only one that is clearly defined.

The incidence of the various etiologic types of pneumonia in a series of cases at Bellevue Hospital is shown in Table I. Over 95 per cent of the cases were caused by the pneumococcus, which emphasizes the importance of this organism in the etiology of this disease.

It is interesting to compare Table I with Table II, which shows a series of pneumonia cases in children (under twelve years of age) distributed according to the infecting organism.

In Bellevue Hospital, since 1920, all of the pneumococcus pneumonias in adults have been classified according to their immunologic types, and the results may be seen in Table III. A corresponding series in children, over a two-year period, is tabulated in Table IV.

Recently, Cooper, Edwards, and Rosenstein reported their work on the separation of the Group IV pneumococci into a number of fixed types. The

TABLE I
BACTERIOLOGIC CLASSIFICATION OF 2,000 CASES OF PNEUMONIA,
BELLEVUE HOSPITAL, 1920-1925*

BACTERIA	NUMBER OF CASES	PERCENTAGE OF INCIDENCE
Pneumococcus	1,913	95.65
Hemolytic streptococcus	76	3.8
Friedlander's bacillus	8	0.4
Influenza bacillus	1	0.05
Staphylococcus aureus	2	0.1
Total	2,000	

*Cecil R. L. Baldwin H. S. and Larsen N. P. Lobar Pneumonia Arch. Int. Med. 40: 253-260, 1927.

TABLE II
BACTERIOLOGIC CLASSIFICATION OF 329 CASES OF PNEUMONIA IN CHILDREN,
BELLEVUE HOSPITAL, 1928-1930

BACTERIA	NUMBER OF CASES	PERCENTAGE OF INCIDENCE
Pneumococcus	308	93.6
Streptococcus viridans	13	4.0
Hemolytic streptococcus	3	0.9
Staphylococcus	5	1.5
Total	329	

TABLE III
INCIDENCE OF PNEUMOCOCCUS TYPES IN LOBAR PNEUMONIA OF ADULTS TREATED IN
BELLEVUE HOSPITAL, 1920-1930*

PNEUMOCOCCUS	NUMBER OF CASES	PERCENTAGE OF INCIDENCE
Type I	1,131	30.9
Type II	850	23.2
Type III	434	11.9
Group IV	1,247	34.1
Total	3,662	

*This series does not include cases admitted during the season of 1925-1926.

TABLE IV
INCIDENCE OF PNEUMOCOCCUS TYPES IN LOBAR PNEUMONIA OF CHILDREN TREATED IN
BELLEVUE HOSPITAL, 1928-1930

PNEUMOCOCCUS	NUMBER OF CASES	PERCENTAGE OF INCIDENCE
Type I	32	10.4
Type II	9	2.9
Type III	10	3.2
Group IV	257	83.4
Total	308	

incidence of these new types at Bellevue Hospital, during the year 1928-1929, in both children and adults, can be seen in Table V. The prevalence of certain of the new types is particularly noted in the children's series.

The importance of an etiologic and type diagnosis in pneumonia cannot be too strongly stressed. Such a diagnosis is really essential to the administration of specific treatment on a rational basis. Furthermore, by knowing what is the infecting organism the doctor has a key to probable complications, and more definite data for his prognosis, in short, he is equipped with a much better general scientific understanding of the case.

TABLE V
INCIDENCE OF TYPES IN PNEUMONIA, BELLEVUE HOSPITAL
Adult Cases, Nov. 1, 1928 to May 30, 1929
Children's Cases, Nov. 1, 1928 to April 30, 1929

PNEUMOCOCCUS	ADULTS		CHILDREN	
	NUMBER OF CASES	PERCENTAGE OF INCIDENCE	NUMBER OF CASES	PERCENTAGE OF INCIDENCE
Type I	75	18.1	14	9.5
Type II	140	33.7	3	2.0
Type III	31	7.5	3	2.0
Type IV	17	4.1	4	2.7
Type V	32	7.7	9	6.1
Type VI	7	1.7	19	12.9
Type VII	16	3.9	5	3.4
Type VIII	3	0.7	1	0.7
Type IX	6	1.4	6	4.1
Types X, XI, XII, XIII	8	1.9	3	2.0
Unclassified	72	17.3	73	49.7
No Pneumococci	8	1.9	7	4.8
Total	415		147	

EXAMINATION OF THE SPUTUM

In all types of pulmonary disease, the sputum is the image of the process in the lung, but a careful examination of this image is required and the findings must be accurately interpreted to be of any value. In a small percentage of pneumonia patients no sputum is procurable. In such cases a throat swab may give the same results. From our viewpoint, the bacteriologic examination of the sputum is the most important, but the histologic findings are also significant, particularly in certain forms of pulmonary disease. If the sputum of patients with diseases of the lung is carefully and repeatedly examined there are sui-

prisingly few instances in which it does not disclose the infecting organism and give a good clue to the process existing in the lung

A great deal of care should be taken in obtaining specimens of sputum for examination. The essential points are to obtain a specimen from the deeper air-passages free from saliva, and to collect it in a sterile container such as can be easily handled, and will be convenient for careful gross examination. In a hospital it is most satisfactory to collect the sputum in a sterile Petri dish. The patient is directed to use the dish only when the sputum is expectorated from the lung. If the patient has not raised the required sputum of his own accord, within one or two hours he is asked to cough deeply once or twice, while lying on his normal side and if this is not effective a throat swab is procured. The specimen should be sent to the laboratory as soon after collection as possible. In case of delay, it should be kept in an ice box or refrigerator until it can be examined.

The gross appearance of the sputum is extremely important and frequently is an invaluable factor in the clinical findings. Examination should be made with a consideration of the following:

- 1 Amount
- 2 Consistency watery, tenacious, mucoid, mucopurulent, purulent, nummular, bloody
- 3 Color colorless, rusty (prune-juice), bloody (fresh blood), yellow, green, brown, black, white, bile colored
- 4 Odor odorless, musty, sweetish, foul
- 5 Particulate material caseous particles, foreign bodies, casts, pneumoliths, broncholiths, Curschmann's spirals

A pneumococcus pneumonia usually produces a characteristic rusty (prune-juice), tenacious sputum. Early in the disease, it may be frankly bloody and in the later stages, as resolution progresses, it may become mucopurulent or occasionally purulent. It is not possible to distinguish the various types of pneumonia by the gross appearance of the sputum alone. In streptococcus pneumonia the sputum is characteristically greenish, and purulent, in staphylococcus pneumonia it is yellowish and purulent, in Friedlander's bacillus pneumonia it is extremely tenacious.

Microscopic examination of direct films of the sputum is an important procedure and should always be included in the laboratory routine. Direct films are best made in the following way. A small particle of the sputum to be examined is placed on the right-hand third of a clean slide which is held in the left hand, and another slide is placed over the sputum. The slides are then pulled apart, leaving a film of sputum on the opposing surfaces. Immediately, both slides are warmed, carefully, over a Bunsen flame, and at the same time, the surfaces covered with sputum are scraped together until the slides have the appearance of ground glass. In this manner an even film is insured.

The films are stained by the Gram or Ziehl-Neelsen method. The microscopic study of sputum supplies information regarding the cellular structure of the specimen and thereby gives some indication of its source. Of chief significance is the information which these studies give regarding the pre-

dominance and nature of the organisms present. Acid-fast organisms are readily distinguished by the Ziehl-Neelsen method. The pneumococci and other pyogenic organisms are best shown by the Gram stain. By the latter method the capsules of the pneumococcus are occasionally detected. However, when a more careful examination of the capsule is required, Miss's copper sulphate method is an excellent one to employ. In some cases the Type III pneumococcus is definitely identified because of its characteristically large capsules. Gentian violet stain is effective for the demonstration of fusospirochetal organisms and is recommended for the examination of sputum from patients with lung abscess and bronchiectasis.

SPUTUM TYPING

The purpose of typing the sputum is to separate the pneumococci into their various immunologic groups, in order to complete the etiologic diagnosis. This type diagnosis has value in prognosticating, but is even more valuable in outlining specific therapy. Pneumococcus immune serum is type specific. It is required of serum therapy that the preparation used should contain antibodies against the particular type causing the infection, and it is equally important that it be administered as early as possible in the course of the disease. With these facts in mind, it is readily understood that the essentials of typing are accuracy, rapidity, and efficiency.

In recent years, at Bellevue Hospital, a procedure has been adopted in which certain features of a number of accepted methods of typing are employed. This procedure is based, primarily, on the use of the mouse for culturing the pneumococcus and separating it from the various normal mouth organisms, and also upon the utilization of the agglutination and precipitation phenomena which occur when the specific immune serum is added to a growth of pneumococci. This combination method is carried out in the following manner:

The sputum to be examined is collected in a sterile Petri dish and sent to the laboratory. The gross appearance is carefully noted and described, and the necessary films are prepared.

The cover of the Petri dish is removed and inverted. The sputum is washed in 2 to 5 cc of sterile physiologic saline solution, in order to free it from saliva and mouth contaminants. A small portion of the sputum, about 1 cm in diameter, is selected and transferred to the Petri dish cover, into which 2 cc of the physiologic saline has been poured. The sputum is then emulsified, first by whipping it with a small applicator, and then by forcing it through a sterile 2 cc Luer syringe, several times. When the mixture is thoroughly emulsified, from 0.5 to 1 cc is retained in the syringe, for mouse inoculation. A white mouse, of good breed, of either sex, weighing from 18 to 22 grams, is used. The injection is made intraperitoneally, preferably directing the needle through the muscles of the thigh and thence to the abdomen, the site of the injection having been carefully sterilized with 95 per cent alcohol.

When sputum is not available, a throat swab is procured by streaking the posterior pharynx with a cotton applicator several times or until the patient coughs. The swab is cultured for two hours at 37° C in 3 cc of beef-heart

infusion broth, PH 7.8, to which 0.15 c.c. of defibrinated blood has been added. At the end of this time 1 c.c. of the culture is injected intraperitoneally into a mouse, and the pneumococcus typing is carried out in the same manner as when sputum is used for the examination.

If the specimen of sputum is sufficient in amount and of the characteristic pneumonia type, the Krumwiede method of typing is carried out. This method is here described.

KRUMWIEDE RAPID PNEUMOCOCCUS TYPING

A portion of sputum (5 to 10 c.c.) is placed in a 15 c.c. centrifuge tube. The tube is set in boiling water for three to five minutes to coagulate the albuminous portion and to free the soluble substance. If the quantity of clear, supernatant fluid which contains the soluble substance is sufficient to complete a precipitation test with the type specific antipneumococcus serum, it is desirable to use it in its undiluted form. In case the quantity is not sufficient (is less than 0.6 c.c.) it is necessary to add a sufficient amount of physiologic saline (not more than 0.5 c.c.) in order to complete the test. If too much saline is added, the soluble substance which is produced in variable quantities by the different types of pneumococci may be so diluted as to prevent a positive reaction. If saline is added, the tube is again placed in boiling water, and is stirred occasionally, for two minutes, in order to completely extract the soluble substance. The tube is then centrifuged at a high rate for ten to fifteen minutes. The supernatant fluid is removed from the tube and is layered, in amounts of about 0.2 c.c., on equal volumes of undiluted diagnostic serums contained in small culture tubes. A test is positive when a precipitation ring forms between the two layers. The tubes used must be clean and perfectly transparent, because the precipitation ring is difficult to distinguish. In order to confirm the results of the ring test, the tube is shaken and incubated for fifteen minutes at 37° C. At the end of this time, in case of a positive finding, a fine white flocculation is noted. The success of this method depends upon (a) obtaining from the patient an ample specimen of pneumonia sputum expectorated from the deeper air passages and (b) the delicacy of the technique employed. In competent hands, this method gives very accurate results. The principle disadvantage is that it can be used in only about 25 per cent of pneumonia cases. When this method is impracticable, or gives negative results, the Sabin rapid method of typing is carried out.

SLIDE AGGLUTINATION TYPING OF SABIN

"From three to four hours after the injection of the mouse, some of the peritoneal fluid is obtained by puncture with a glass capillary. A glass slide is marked off into four parts, and a minute drop of the peritoneal fluid is expelled on each one of the four partitions. The first is smeared with saline for control, and the others with a loopful of 1:10 dilution of Type I and of Type II, and a 1:5 dilution of Type III diagnostic serums respectively. These dilutions of serum are chosen largely to eliminate group agglutinins. The smears are made thin, allowed to dry, and fixed by passing the slide through a

flame, they are then stained from twenty to thirty seconds with a fuchsin solution (10 cc saturated alcoholic solution of basic fuchsin plus 90 cc of water) or any other available stain. The stain is washed off in water or 20 per cent copper sulphate solution, and the smears are examined with the oil immersion lens. If a specific agglutination reaction is observed in one of the smears with diagnostic serum, the organism is of that type. If no reaction occurs in any of the smears, and numerous pneumococci are clearly seen, a diagnosis of Group IV is suggested. When it is desired to know whether the organism is one of the fixed types of Group IV (especially those for which concentrated antisera are available), a similar procedure is carried out with the corresponding diagnostic sera. Bacteria in the sputum which are not pneumococci as well as avirulent forms of pneumococci may occur in clumps in the peritoneal exudate, but these differ in appearance from those produced by specific agglutination, they can be distinguished further by their occurrence in the saline control smear as well. Unless a fresh sample of sputum is used, many of the organisms will have undergone autolysis, and therefore more time must be allowed for growth. Since the mouse is not killed, another typing can be done if the first one should show insufficient organisms, and after death of the mouse, the type may be confirmed. In the case of Type III, sufficient organisms are usually present even two hours after injection. The appearance of the specific reaction with Type III differs somewhat from that obtained with other types of pneumococci, primarily on account of the larger size of the capsule, the organisms are further apart in the agglutinated clumps which occur in mucoid strands."

If a positive finding has not been obtained by either the Krumwiede or the Sabin method of typing, after the mouse has died, the macroscopic agglutination and precipitation tests are carried out on the peritoneal exudate in the following manner:

The peritoneal cavity is opened with sterile precautions, and a culture is made by streaking some of the exudate on a blood agar plate by means of a platinum loop. At the same time, films are made from the exudate. Next, the peritoneal exudate is washed with 5 cc of sterile saline solution by means of a sterilized glass dropper. The washings are placed in a sterile centrifuge tube. Before the body of the mouse is discarded the thorax is opened and a blood agar plate is streaked with a loopful of the heart's blood.

Typing is continued by centrifuging the peritoneal washings, first, at a low speed, in order to throw down the cells and fibrin, and then, after transferring the supernatant fluid to another sterile centrifuge tube, at a high speed, in order to separate the organisms. After this procedure, the supernatant fluid is transferred to a tube to be used for the precipitin test. The bacterial sediment is resuspended in about 2 to 3 cc of normal saline solution, a sufficient quantity being used to give the appearance of an eighteen-hour broth culture of pneumococcus. This bacterial suspension is used for the agglutination test.

For these tests small culture tubes (3 by $\frac{3}{8}$) are arranged in two rows in racks for placing in the water-bath. One row of tubes is used for the agglutination test and the other, for the precipitation test. For the former, 0.2

c.c. of a 1:10 dilution of each diagnostic serum is transferred to the tubes with a 1 c.c. pipette. For the latter, the same procedure is carried out using undiluted diagnostic serum.

The bacterial suspension is used for the agglutination test, and the centrifuged supernatant fluid is used for the precipitation test, for both 0.2 c.c. amounts are added to the tubes already arranged. The tubes are then incubated for two hours in a water-bath, at 37° C. or for two hours at 56° C. At the end of this time the tubes are carefully examined for clumping and flocculation, indicative of the specific type.

Confirmation of Type—The type obtained by any of the aforesaid methods is confirmed by typing the pneumococci cultured from the heart's blood of the mouse. After streaking the blood agar plate with the heart's blood, at the time of the mouse autopsy, the plate is incubated for eighteen to twenty-four hours at 37° C. At the end of this time a discrete colony of pneumococci is transferred, with a platinum loop to 9 c.c. of beef-heart infusion broth containing 5 per cent of defibrinated blood. This culture is incubated for twelve to twenty-four hours and then 0.5 c.c. of it is added to 9 c.c. of plain broth, and, again, incubated for the same length of time. This broth culture of pneumococci is typed by the agglutination method outlined above and at the same time, the organisms are tested for bile solubility by adding 0.3 c.c. of the culture to 0.1 c.c. of whole ox bile. A solution of pure sodium desoxycholate one part to 500 parts of culture may be used instead of the whole bile. This method of typing gives exceedingly accurate results which are valuable in confirming those obtained by the more rapid methods.

BLOOD CULTURES IN PNEUMONIA

The taking of blood cultures in pneumonia is an important diagnostic and prognostic procedure. The etiologic diagnosis is usually first made by the sputum typing. However, finding the same organism in the blood is important confirmatory evidence. Perhaps, even more significant from a diagnostic point of view, is the value of this procedure in recognizing complications of pneumonia. Of these complications, septicemia is one of the most frequent. Many of the others, such as meningitis, endocarditis, and pericarditis occur as sequelae of the septic condition rather than of the pneumonia itself. In prognosis the determination of the degree of septicemia is more elucidating than the mere discovery of its presence.

The mortality in pneumonia patients whose blood contains more than 100 organisms per cubic centimeter is practically 100 per cent. On the other hand, the outlook is by no means hopeless in the presence of only a few organisms particularly when found early in the disease.

Blood cultures are best made at the patient's bedside. Three cubic centimeters of blood is removed, under sterile precautions, with a 5 c.c. syringe. Of this, 1 c.c. is inoculated into 9 c.c. of plain broth and the remaining 2 c.c. is used for two agar pour plates. These cultures are then sent to the laboratory and incubated, at 37° C., for twenty-four hours. At the end of this time, the colonies on each plate are counted and the broth is typed by the agglutination method, as described above care being taken to prevent shaking of the tube

before the suspension is removed. Negative cultures are allowed to remain in the incubator for four days before the final report is given, and the tubes and plates are discarded.

CHEST FLUID, SPINAL FLUID, AND JOINT FLUID CULTURES IN PNEUMONIA

Empyema, meningitis, and septic arthritis are not rare complications of pneumonia, particularly following pneumococcemia. Whenever fluid is removed from the chest, spinal canal, or joint, it should be submitted to careful bacteriologic study. The appearance of these fluids is usually sufficient to diagnose the particular complication. However, knowing the number and variety of organisms present gives additional significant information, particularly in confirming the results of the original typing.

The bacteriologic study of the fluids should be complete. It is well, first of all, to make films and stain them by the Gram method. The fluid should be streaked on a blood agar plate and 0.5 to 1 cc injected into a mouse. The pneumococci are typed from discrete colonies on the plate or from the mouse exudate, in the manner described under sputum typing. When pneumococci are present in large numbers in any of the body fluids they may be typed directly by the Sabin slide agglutination method.

COMMENT

Perhaps the greatest advance in the practical laboratory procedure in pneumonia within the last few years has been in the modification of bacteriologic methods to give more accurate and more rapid results. The use of the Sabin rapid method of typing makes it possible to obtain an accurate type diagnosis on the day the patient is first seen (before its adoption, there was always a delay of from eighteen to twenty-four hours). The chief practical advantage of this rapid typing is that a type diagnosis can be made as a preliminary to the consideration of specific therapy.

In the early days of serum therapy, one of the major criticisms was that a large number of patients were subjected to the inconvenience and expense of serum injections and later were shown to have a type of infection that was not compatible with the serum administered. In addition to the improvements in typing, there have also been improvements in blood culture methods. For instance, it has been found a distinct advantage to withdraw a small amount of blood, such as 3 cc, for which a small needle and a small syringe are adequate. With skillful technique this operation causes practically no discomfort to the patient, and if repeated at regular intervals becomes one of the most valuable aids in prognosis.

Shortly after Sabin described his rapid method of typing, other investigators reported modifications which in their own hands gave comparable results. Armstrong suggests the examination of an unmixed and unstained mixture of mouse exudate and diagnostic serum in order to observe the agglutination of the organisms. Calder uses the hanging-drop method and a vibrating machine which he has devised in order to obtain definite and rapid agglutination. Each of these three methods is dependent upon finding a sufficient num-

ber of organisms in the peritoneal exudate. Hence, they are all unsatisfactory in certain cases. The most important factor in the operation is experience with the method used.

During the past few years, pneumonia has been the subject of extensive investigation, but most of the discoveries have not yet reached the stage of practical significance. Perhaps the most important advance toward the solution of this problem has been the chemical fractionation of the pneumococcus. This information has recently been applied by Avery and Dubos to the development of an enzyme which splits the carbohydrate fraction of the Type III pneumococcus, and by so doing destroys the toxicity of this organism. Already these scientists have been able to protect mice against the Type III pneumococcus infection, and it is probable that this agent will be of value in the treatment of pneumonia.

The artificial transmutation of types is another achievement of recent years. Griffith and Dawson have described methods of transforming pneumococci from one type to another, both *in vivo* and *in vitro*. These discoveries add a great deal to our knowledge of the pneumococcus and the infection which it produces. At present, however, we have no realization of their ultimate value.

The development of the modern treatment of pneumonia has passed through various stages. The first might be called the prebacteriologic era, the second, the pretyping, the third, the pierapid typing, and the fourth, that of the present day. Very few doctors are now practicing in the prebacteriologic era, but an isolated case occasionally comes to our notice. For example, a certain patient is known to have been treated as having lobar pneumonia, followed by delayed resolution, and after six weeks of care at home, surrounded by children, the consultant discovered numerous tubercle bacilli in the sputum. This unfortunate patient certainly was handled according to prebacteriologic methods.

The majority of pneumonia patients today are not given the benefit of typing even when the advantages of type diagnosis are so striking, as shown by the following example. Two pneumonia patients with the same amount of lung involvement and the same degree of toxemia are seen late in their infection. Assume that one is a Type I, with a negative blood culture, and the other, a Type III, with a positive blood culture. The Type III patient will almost certainly die. The Type I has a good prognosis, and, if given the benefit of an efficient therapeutic agent, such as concentrated serum, will almost certainly recover.

In the up-to-date management of pneumonia, the sputum should be carefully examined and rapidly typed as a guide to prognosis and to insure the prompt administration of serum in types in which it has been found efficacious. Blood cultures and other laboratory procedures should be instituted in order to recognize early the presence of septicemia and other complications. In short, it is evident that the modern treatment of pneumonia is dependent upon the utilization of the latest bacteriologic and immunologic methods.

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(Symposium to be continued in April issue)

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EDITORIAL

The Symposium

A symposium on clinical bacteriology is of particular significance and value at the present time when the news dispatches in featuring the discoveries in the field of medical bacteriology are usually guilty of a hyperbole. The press when using such descriptive statements as "The most important step in medical bacteriology since Pasteur", "This discovery overthrows all of the well-founded tenets of this branch of the natural sciences", ad nauseam, is only too frequently repeating the hopes of the worker rather than an evaluation of the data by the investigator's peers.

Such projected achievements as "The sterilization of a city water supply by the occasional addition of a test tube full of a tiny destroyer of germs, the bacteriophage," may erase for a moment from the minds of the public health workers the worries of swimming pool control, but the statement "The invisible virus of encephalitis is ever present among us as the visible hay bacillus," only causes consternation in the ranks. Again one reads "The causative agent

of influenza is easily unmasked by a diet of hog's intestine," which is all the more confusing when the reader remembers an announcement of a short time before, "The agent of influenza masquerades as a pleomorphic streptococcus"

The effect of such bombastic circumlocutions, which ordinarily in due course of time prove to be plain rhetoric, on the public and the routine laboratory technician cannot be overestimated. The former visualizes the sudden conquest of all the afflictions of mankind while the latter only too often begins to doubt the value of the tried and true procedures and attaches a subterfuge for all uncertainties and failures.

Recent investigations on life cycles, mutation, dissociation, virus stages, sexual reproduction, filterable forms etc., in bacteria are of extreme academic interest but their significance must be thoroughly understood and their influence on diagnostic technique carefully demonstrated before they are introduced into routine methods, lest pandemonium result. To insist on a demonstration of value is often extremely irritating to the exponent of a new theory and some accept to follow lest the exponent hurl the facile sentimentality, "The nature of your background and training incapacitates you for sympathy with modern and progressive thought," when as is usually the case, the poor worker only happens to be one of those unbiased readers who calmly surveying the evidence is astounded perhaps more by the easy saltations in the enthusiast's judgment and interpretation than in the bacterial form or phenomenon he is describing.

It is a pleasure on perusing the various contributions, so finely diversified, to find the cautious interpretations placed on the results of the separate procedures by the several writers, authorities in their own fields, yet the confidence in the results when the methods are carefully followed.

M H S

Program of the International Congress on Asthma

At Mont-Dore, France

June 4 and 5, 1932

- 1 Evolution of Our Knowledge of Asthma—Fernand Bezancon
- 2 Pathological Physiology of the Asthmatic Crisis—Abram
- 3 Anaphylaxis in Asthma—P. Vallery-Radot
- 4 Nervous Factor in Asthma—Etienne Bernard
- 5 The Liver and the Endocrine System in Asthma—Cordier
- 6 Etiology and Pathology in the Treatment of Bacterial Asthma—Haibe
- 7 The Role of the Nasal Ganglion in the Production of Asthma—Halphen
- 8 Respiratory Equivalents of Asthma—Bourgeois
- 9 Morbid Relationships of Asthma—Andre Jacquelin
- 10 Etiology, Pathogenesis in Treatment of Infantile Asthma—Lesne
- 11 Diagnostic Signs and Evolution of Infantile Asthma—Pehu
- 12 Treatment of the Asthmatic Crisis—Joltrain
- 13 Treatment of the Asthmatic Terrain—Lucien De Gennes

- 14 Hydro-Mineral Treatment of Asthma—Villaret and Besancon
- 15 Physiotherapy in Asthma—Biancani
- 16 Surgical Treatment of Asthma—Jeniche and Fontaine
- 17 Mont-Dore and Asthma—J Galup

The above contributions are from among the leading French physicians interested in asthma. The following are on the program as representatives of foreign countries:

- Professor Prausnitz (Germany)
- Mac Dowall (England)
- Clementino Fraga (Brazil)
- Mariano Castex (Argentina)
- Maranon (Spain)
- Longcope (United States)
- Storm Van Leeuwen (Holland)
- Frugoni (Italy)
- Danielopolu (Roumania)

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Erratum

Through an error in make-up, abstract material that had been previously published was reprinted in the February issue.

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SPUTUM EXAMINATION IN PULMONARY TUBERCULOSIS*

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THE topic indicated in the title of this paper seems hardly worthy for a journal article. Such elementary discussion should really be confined to textbooks. But in spite of the increased emphasis put on tuberculosis teaching the following quotation is still a fair statement of facts. This may justify the subsequent pages.

"A plea for the general tightening up of the technic of collection and examination of the sputum may not be out of place here. The character of many specimens received in laboratories and their slipshod handling afterward are enough to make one wonder as to just how trustworthy the average of reports may be. I have seen senior house-officers in first-class hospitals begin with the sputum in a narrow-neck dark bottle, then introduce the platinum loop blindly, withdraw whatever mucus clung by chance to the loop smear this on a slide, stain and, after a rather casual survey—search would be altogether too inaccurate a term—report the specimen as negative. In other words there were violations of good technic at every single stage of the examination." (Krause²⁹)

1 COLLECTION OF SPUTUM

The first and a very important step in the examination of sputum for tubercle bacilli is the proper collection of sputum. It should not be necessary to mention the need for bacteriologic cleanliness of the containers but since sterilization does not necessarily destroy the morphologic entity of tubercle bacilli it is preferable to use containers for sputum specimens only once such as paraffinized cardboard boxes or ice cream boxes.

From manifold experience it seems imperative to emphasize the necessity of properly instructing the patient. Too many times the patient's assurance

*From the Laboratories of the Desert Sanatorium and Institute of Research.

that he does not expectorate is accepted with facile acquiescence, although the patient—frequently hardly initiated in the customs and parlance of things medical—is wholly ignorant of the meaning and the clinical significance of such terms as “sputum” and “expectoration.” A simple and patient instruction—if need be in the vernacular rather than in medical terms—will often be productive of ample specimens the sources of which the patient had been quite unaware. The patient must and will learn to differentiate between true sputum and nasopharyngeal drippings and saliva, and he can be educated to avoid sending along with his sputum such foreign bodies as are of no medical interest. In many instances it is necessary to have sputum collected over a number of days.

In young children, it is usually impossible to obtain sputum. Tubercle bacilli may, however, be found in smears from pharynx, tonsils, or larynx, in feces and in gastric contents. The gastric contents, preferably collected in the morning before breakfast, by gastric lavage, are treated by concentration methods exactly in the same manner as sputum. (Aimand Delille and Vibert²)

The methods for demonstrating tubercle bacilli in feces are essentially the same as those for sputum, discussed later. Animal inoculation should be used whenever direct smears show no bacilli, since cultural methods are somewhat less successful with stools than with sputum.

2 PREPARATION OF DIRECT SMEARS

In every complete sputum report, the gross appearance of the specimen should be mentioned. Although there is no sputum characteristic in its gross appearance for tuberculosis, the nature of a specimen may frequently form an important link in the clinical evidence, diagnostically or prognostically. Any special gross feature of the sputum should be carefully reported. Layer formation, especially in association with basal lesions, is of great diagnostic significance. The relative amount of pus, as seen macroscopically, must be noted. Much pus in the absence of tubercle bacilli throws the weight against pulmonary tuberculosis, here again correlation with clinical and roentgenologic findings goes a long way toward establishing a diagnosis. When the diagnosis of pulmonary tuberculosis is established, fluctuations in the relative amount of pus are prognostically significant. And may it be emphasized here, that in pulmonary tuberculosis one need not search for the so-called pyogenic organisms to explain the presence of pus in the sputum, the tubercle bacillus is itself a pyogenic organism. So-called “secondary infection” plays undoubtedly an insignificant rôle. A true secondary infection of the tissue is exceedingly rare, while secondary infection of the *necrotic contents* of cavities is common, but apparently without clinical significance. While emphasizing the often neglected need of reporting the gross appearance of sputum, it should be remembered that no description can substitute for the visual impression that the clinician should insist upon procuring for himself at frequent intervals. For the laboratory it must be emphasized again and again, that any kind of sputum may contain tubercle bacilli, be it ever so small in amount, or be it ever so “innocent-looking,” watery clear, like saliva. Therefore no specimen may ever be discharged without careful bacteriologic study. Occasionally patients with the clinical evidence of pulmonary tuberculosis are seen who produce practically no sputum,

but who have an occasional hemorrhage. In such cases the expectorated blood should be examined for bacilli, the search will be successful in a number of instances. The amount of twenty-four-hour specimens should be reported by weight and not by volume.

It is a good rule to start every sputum examination with a study of a fresh unstained smear. This may not be feasible where a large volume of routine work has to be done. Since the results of fresh smear studies are chiefly of importance in nontuberculous pulmonary disease these findings will not be discussed here with the exception of the demonstration of elastic fibers. Although special methods for their demonstration are advocated they can easily be found in the fresh unstained smear. The presence of elastic fibers indicates destruction of pulmonary tissue regardless of the etiologic agent. If they are present in sputum in which careful complete and repeated studies fail to demonstrate tubercle bacilli the probability is very great that tuberculosis is not the cause of the destructive pulmonary lesion.

In preparing smears for the detection of tubercle bacilli three points frequently neglected are particularly important:

1. Select with utmost care a particle of sputum
2. Make thin and even smears
3. Make a very light counterstain

Point 1. If this step is performed with care and understanding a great deal of time will be saved. Since bacilli are not evenly distributed but are massed together in purulent or cheesy particles, such particles must be selected for examination. If the specimen is spread out in a Petri dish and viewed against a black background the proper selection of particles becomes an easy matter.

Point 2. Unless this precaution is taken, decolorization is difficult and bacilli may remain hidden under cellular or mucoid material.

Point 3. It is my experience that this rule is most frequently violated not only by pseudotechnicians, but by expert workers too, and by competent textbooks. Loeffler's methylene blue, diluted with water 1:80 and applied for five to ten seconds gives a sufficiently distinct counterstain without obscuring tubercle bacilli.

A brief discussion of stains for tubercle bacilli follows.

Bacterial Stains.—Most methods use basic fuchsin. Herman^{15, 19} advocated crystal violet, and his method is indeed very satisfactory for tissue stains but it has no apparent advantages over the time-honored fuchsin stains for sputum smears.

Mordants.—The most commonly used mordant is carbolic acid. In Herman's method ammonium carbonate is substituted.

Decolorizing Agents.—The most satisfactory decolorizing agent is a 3 per cent solution of concentrated hydrochloric acid in 95 per cent alcohol. Nitric acid which is advocated in a number of methods is less satisfactory because traces of nitrous acid may decolorize tubercle bacilli (Krause quoted by Willis)²⁰ Weichselbaum, in a method modified by Gabbett²¹ used decolorization and counterstaining agent in one solution. This procedure although somewhat speedier

cannot be recommended because the process of decolorization cannot be controlled properly Konich⁸ reduced the fuchsin to its colorless leucobase by the use of sodium sulphite This method has advantages when, for some reasons, alcohol is difficult to obtain, but it is somewhat more cumbersome since the sodium sulphite solution must always be freshly prepared

Counterstain—Methylene blue, as used in the Ziehl-Neelsen method is quite satisfactory because it produces sharp definition and good contrast It should, as any other counterstain be used in such a dilution, as to stain the cells only a faint blue If the counterstain is applied too heavily tubercle bacilli may be obscured by it, and even if this be not the case the bacilli are much less readily seen Spengler⁴⁸ advised picric acid as counterstain Since, however, picric acid produces only a very faint yellowish background without definitely outlining the cellular elements, the eye fatigues much more quickly examining slides stained by this method A further disadvantage of this method is the fact that it does not enable the observer to recognize cellular elements and other bacteria Brilliant green, in proper dilution, is as good a counterstain as methylene blue

The literature on stains for tubercle bacilli is immense, new methods and modifications have been advocated ad infinitum, but it is a highly significant fact that whenever comparative studies are made, the Ziehl-Neelsen method, with or without minor modifications, remains victorious as an accurate and simple method (See for example Cooper, 1926) Inquiring in many laboratories, one finds that the vast majority of them uses the Ziehl-Neelsen method, although many have, as has the author, given fair trials to other methods A recent modification seems to have slight though definite advantages in our experience, without complicating the procedure This is Cooper's modification which yields excellently defined preparations

The original Ziehl-Neelsen method, with rather insignificant deviations in the preparation of the staining solution and Cooper's modification, follow

I Ziehl Neelsen

1 Saturated solution of basic fuchsin

Basic fuchsin	20 cc
Alcohol, 95 per cent	100.0 cc

2 Carbofuchsin solution

Saturated solution of basic fuchsin	10.0 cc
Carbolic acid, aqueous 3 per cent	90.0 cc

Steam three minutes, wash, decolorize in 2.5 cc hydrochloric acid plus 97.5 cc 95 per cent alcohol Wash, counterstain with Loeffler's methylene blue diluted with water 1:80 for five to ten seconds, wash, dry

II Ziehl Neelsen (Cooper's modification)⁴

Flood the slide with fresh carbofuchsin to which 3 cc of 10 per cent sodium chloride per 100 cc is added, steam for four minutes and allow to cool until the precipitate forms Wash with tap water Decolorize with acid alcohol (5 cc nitric acid, sp gr 1.42 plus 95 cc of 95 per cent alcohol), wash, two minutes 95 per cent alcohol, wash, counterstain with 1 per cent brilliant green in 1:10,000 sodium hydroxide for one minute, wash, dry (For reasons previously mentioned, 2.5-3 per cent hydrochloric acid in 95 per cent alcohol is a more satisfactory decolorizing agent)

Since Much's work on granula was published a number of staining methods have been elaborated with the purpose of staining both the classical acid-fast rods and Much's nonacid-fast granules It is still very doubtful whether Much's

granules the existence of which is not doubted can be differentiated by these methods in sputum smears from other coccoid bodies. They can at the present time not be accepted as a diagnostic criterion. But since a considerable theoretical interest is attached to them recently revived by the work of Sweeney and of Kahn, it may be mentioned that the method of Weiser and the less cumbersome method of Kiefer have yielded very satisfactory results in my hands.

*Kiefer's Stain*²

Carbolfuchsin	4 parts	} by volume
Carbol methylviolet	3 parts	

If this mixture shows a metallic scum add 95 per cent alcohol drop by drop until the scum disappears.

Fixing solution

Iodine	1 gm
Potassium iodide	2 gm
Distilled water	100 cc

Decolorizing solution

Concentrated hydrochloric acid	10 cc
Alcohol, 95 per cent	50 cc
Acetone	40 cc

Counterstain

1 per cent aqueous methylene blue

Stain while heating $\frac{1}{2}$ to $\frac{3}{4}$ minute, rinse, iodine solution 1 to 1 $\frac{1}{2}$ minutes, rinse, decolorize $\frac{3}{4}$ to 1 minute, rinse, counterstain.

A number of staining methods have been devised to stain differentially both tubercle bacilli and elastic fibers in the same preparation. The two methods mentioned below fulfill this requirement fairly satisfactory, but it is doubtful whether they are full substitutes for a careful search for elastic fibers in the unstained preparation.

1 *Pappaport and Ellison*³—Weigert's elastica stain three to five minutes, rinse, decolorize to a faint pink with 3 per cent hydrochloric acid in 95 per cent alcohol, rinse, counterstain with aqueous methylene blue.

2 *Jessen*⁴ uses the Ziehl-Neelsen stain and counterstains for one or two minutes with

Hematoxylin,	10 gm
Lithium carbonate saturated solution,	10 cc
Absolute alcohol,	200 cc
Distilled water,	200 cc

Then rinse, cover for a few seconds with 28 per cent ferric chloride, rinse, dry.

In accordance with what was said in regard to the gross appearance of sputum, the microscopic reports should mention not only the presence or absence of tubercle bacilli, but also the type of cells seen (leucocytes lymphocytes epithelial cells), their relative number and the presence of nonacid-fast microorganisms. This latter observation is clinically more significant than cultures for secondary microorganisms. It is obvious that a properly prepared smear will give a truer picture of the composition of the sputum flora than cultures in which primarily insignificant organisms may outgrow the truly predominant type.

3 CONCENTRATION METHODS

When examinations of direct smears fail to demonstrate tubercle bacilli, further studies should be made on the sputum since rather large numbers of

bacilli may escape detection by the simple smear method. Corper has estimated that more than 100,000 bacilli must be present per c.c. of sputum for their detection on smear preparations. It should become a routine procedure to concentrate each suspected specimen (if negative by direct smears), to culture it, and inject it into a guinea pig if the preceding method has failed.

The first concentration method seems to have been reported by Biedert¹³, he boiled the sputum with a liberal amount of a 0.2 per cent solution of sodium hydroxide. Muhlhauser,¹⁰ and later Czaplewsky and Hensel¹⁴ reported encouraging results with modifications of Biedert's original method.

Uhlenhuth and Nylander,¹⁵ studying the bacteriolytic action of antiformin, a mixture of sodium hypochlorite and sodium hydate, found that antiformin kills bacteria but not tubercle bacilli, and that it destroys other organic material, such as cells. They recommended antiformin for the preparation of sputum or other contaminated material for the isolation of tubercle bacilli in pure culture. Soon, however, this procedure was found exceedingly useful for the bacterioscopic demonstration of tubercle bacilli.

The following procedure can be recommended. About 10 c.c. of sputum is mixed with a 20 per cent antiformin solution to secure an approximate final concentration of 10 per cent antiformin. For thin, watery sputa this concentration should be decreased, for heavy tenacious specimens, it should be increased. The sputum antiformin mixture is incubated at 37° C. for about half an hour, during which time it must be shaken several times, or it may be kept in a shaking machine for an equal length of time. The antiformin will transform the sputum into a clear, homogeneous fluid, destroying and dissolving all formed elements, with the exception of tubercle bacilli. The time necessary for complete digestion varies with different specimens. If the preparation is made for the sole purpose of bacterioscopic examination, the period of contact between sputum and antiformin does not matter, as long as it is extended enough to effect a complete homogenization, but if one wishes to isolate tubercle bacilli in pure culture, the shortest period effecting complete clearing is the optimal one. After clearing is complete the mixture is centrifugalized at high speed (preferably after the addition of an equal amount of water—or alcohol if no cultural studies are intended—in order to reduce the specific gravity). The supernatant is discarded and the sediment is neutralized with hydrochloric acid. The sediment is then ready to be spread on slides and stained. Since the sediment washes off readily in staining, it should be fixed to the slide with a 10 to 20 per cent solution of egg albumen.

The most successful application of Biedert's principle, the homogenization of sputum by alkalis, is the procedure published by Petroff¹⁶, it has found wide acceptance. In Petroff's method the sputum is mixed with an equal amount of 4 per cent sodium hydroxide. Otherwise, the procedure is the same as with antiformin. Dilution before centrifugalization and fixation with egg albumen are unnecessary.

Sweany¹⁷ has worked out a procedure, using the alkali digestion of sputum, which seems to be very useful for work on large scale.

4. CULTURAL METHODS FOR THE DEMONSTRATION OF TUBERCLE BACILLI

a. The Preparation of the Sputum—In recent years cultural methods have been improved to a sufficient degree to play a significant rôle in the demonstration of tubercle bacilli in sputum. Two factors decide the success of cultural methods. (1) The preparation of the sputum in such a way that tubercle bacilli are concentrated within a relatively small amount of material without affecting

their vitality, and at the same time killing other microorganisms as completely as possible, and (2) suitable media

The laborious washing method of Kitasato²⁹ which yielded very inconstant results is now of only historical interest Uhlenhuth's antiformin and Petroff's sodium hydroxide methods were for a number of years the most successful procedures for concentrating tubercle bacilli without much impairment of their viability and for killing contaminating organisms In recent years these methods have been rivaled if not surpassed by acid digestion as advocated by Lowenstein³⁴ and Summowski³⁵

Lowenstein's original procedure however cannot be recommended because he used too high a concentration of acids Hohn² treats the sputum with ten to twelve per cent sulphuric acid for twenty minutes using 1 - 2 cc sputum and 10 cc sulphuric acid After centrifugalization the sediment without neutralization is seeded on Lubenau's medium Other authors recommended further reduction of the acid (Corpei 6 per cent) or the use of a 3 to 6 per cent hydrochloric acid or (Corpei³¹) 5 per cent oxalic acid It has been our experience that we get somewhat better results with acids and after extended trials we prefer to use a 3 volume per cent hydrochloric acid For bacterioscopic work, the sodium hydroxide method is preferable, because it produces a smaller amount of sediment and hence a more effective concentration of tubercle bacilli

Petroff's sodium hydroxide method deserves preference over Uhlenhuth's antiformin method because of its greater simplicity and because it causes probably a smaller percentage of mortality of tubercle bacilli It is important to find the optimal time for digestion not too short, because the contaminating organisms are not killed, not too long because too many tubercle bacilli become sufficiently damaged to fail growing on artificial media In accordance with Sweany and Evanoff³² I prefer using a 3 instead of a 4 per cent sodium hydroxide solution for cultural work The technic is identical with that for the bacterioscopic concentration method previously mentioned, of course, throughout the whole procedure strict aseptic precautions must be maintained The neutralized sediment is planted rather thinly on the slants, the tubes are then sealed with paraffine or sealing wax and a small needle puncture is made through the seal to provide a free supply of oxygen

b Culture Media for the Isolation of Tubercle Bacilli—In the choice of culture media for the isolation of tubercle bacilli it must be kept in mind that not all media on which tubercle bacilli in pure transplants grow well are suitable for isolation Quite generally it can be said that liquid media are unsuitable, and that most agar media are unsuccessful, the same seems to be true of all so called synthetic media

The number of media advocated for isolating tubercle bacilli is so great that it is impossible to mention them all, especially since many are but modifications of previously tried and recommended media

In the preparation of culture media, the following substances are used successfully Serum potatoes, glycerol, eggs, milk Serum media were used by Koch in his first studies²⁷ potato media were recommended by Pawlowsky⁴² (1888) and found particularly suitable in connection with glycerol, the use of which was first recommended by Nocard and Roux⁴⁰ (1888) The British Royal Commis-

sion recommended that the potatoes be soaked in a sodium carbonate solution and Matzuschita¹⁰ (1899) showed by comparative experiments that tubercle bacilli grow better on potatoes that are slightly alkalinized. Eggs, particularly egg yolk, were added to nutrient media by Capaldi¹¹ (1896) and highly recommended for the growing of tubercle bacilli, while media the chief component of which is egg were introduced by Dorset¹² (1902) before he knew of Capaldi's previous studies and by Lubenau¹³ (1907). Matzuschita¹⁰ substituted milk for broth in a gelatine-glycerol medium. Although, as will be evident from the brief historical review just presented, all recent culture media are but elaborations and modifications of principles well established by the turn of the century, the isolation of tubercle bacilli from contaminated material, has, due to the work of the last few years, advanced from a complicated and unreliable method, used almost exclusively for scientific study, to an easy and highly dependable technique in clinical-pathologic routine.

Some of the apparently most useful media are enumerated below. As far as possible, the original prescriptions are given. I would emphasize, however, that all the media which require inspissation, the several varieties of egg media, yield, in our experience considerably better results when instead of the repeated inspissation as prescribed, they are kept in the Arnold sterilizer just long enough to cause coagulation. This brief sterilization yields only a very occasional contaminated tube. It is necessary, of course, to proceed as aseptically as possible in the preparation of the media. It should further be pointed out that the usual "overnight in the incubator" is a totally inadequate sterility test, media containing eggs, milk, or potatoes should be kept in the incubator for not less than three days.

1 *Lubenau's Egg Medium* (1907).—Three parts of well mixed whole eggs are added to one part of 5 per cent glycerol nutrient broth. This mixture is tubed and inspissated for two or three hours at 90° C.

Hohn¹⁴ emphasized the necessity of adding a small amount of glycerol broth to each tube. He further recommended that some hemoglobin be added to the medium, in the following way. Sterile blood clots are pressed through a sterile wire screen, the drippings are centrifuged to remove the serum. The blood is then hemolyzed by the addition of an equal amount of sterile distilled water. This hemoglobin solution is added in the amount of 2 per cent to the medium before tubing. Quite recently, Hohn¹⁵ suggested further modifications, in this publication, his present procedure is reported in minute details.

2 *Petroff's Gentian Violet Egg Medium* (1913).—A veal or beef infusion is prepared in the usual way, using 1000 gm. of meat with 1000 c.c. of a 15 per cent solution of glycerol in water. Eggs are mixed and filtered through gauze under sterile precautions, and one part of infusion is mixed with two parts of eggs. To each liter of this mixture is added 10 c.c. of a 1 per cent alcoholic solution of gentian violet. After mixing well, the medium is filtered through gauze, tubed, and inspissated as follows. First day at 85° C. until coagulated, second and third days at 75° C. for one hour.

3 *Sweany's Veal Egg Glycerol Milk Medium* (1928).—This medium is prepared exactly like Petroff's medium, with the exception that the meat infusion is prepared with sterilized milk instead of with water, and that no dye is added. The milk is sterilized in live steam on two successive days for forty five minutes.

Sweany recommends that sterilized cream, 10 per cent, be substituted for the glycerine in the above medium for the cultivation of bovine bacilli.

4 *Glycerol Potatoes (Corper's Modification)* (1928).—The usual halved potato cylinders are soaked for one or two hours in a 1 per cent aqueous solution of anhydrous sodium carbonate containing 1:75,000 crystal violet, then they are wiped off with a clean towel, put

in culture tubes to which 15 c.c. of a 5 per cent glycerol broth is added, and autoclaved at 15 pounds pressure for at least thirty minutes

5 *Lowenstein's*^{21a} *Modification of Fqq Medium* (1931) —The following solution is prepared

Monopotassium phosphate,	10 c.c.
Sodium citrate,	10 c.c.
Magnesium sulphate,	10 c.c.
Asparagin,	30 c.c.
Glycerol,	600 c.c.
Distilled water,	1000.00 c.c.

To each 150 c.c. of this solution are added 6 gm. potato flour and 12 c.c. glycerol. This mixture is boiled while stirring for fifteen minutes and kept at 56° C. for one hour. Then 4 eggs and 1 egg yolk are added and 5 c.c. of a 2 per cent Congo red or malachite green solution. After thorough mixing, the medium is filtered through sterile gauze, tubed, and inspissated on two subsequent days at 80 to 85° C. for two hours.

Lowenstein claims that peptone inhibits somewhat the growth of tubercle bacilli, and he devised therefore, this peptone-free medium.

An egg yolk agar (a modification of Capaldi's medium) has recently been recommended by Herrold.²⁰ His first studies appear to be promising, but this medium has not as yet stood the practical test.

The choice of a concentration method and of a suitable medium is still much discussed. Each method and each medium has its advocates, the most emphatic advocate being usually the originator of the particular procedure. A careful study of the literature leaves one undecided as to the respective merits of each procedure.

We have tried all methods and media mentioned in this review and a good many more. We have used alkalis and acids in widely varying concentrations, we have made our own modifications. If we present here the procedure which we employ at the present time, it is not because we believe that it is better than any other one or because we think it is final. It is because so far, it has yielded the best results in our hands and because we can say, by well-controlled series of examinations, what this procedure will do. We should like to recommend it for further trial, and we hope that further experience will improve it.

Our Procedure for Isolating Tubercle Bacilli —Approximately 10 c.c. of sputum and 10 c.c. of 3 per cent (by volume) of hydrochloric acid are thoroughly mixed in a sterile, stoppered 50 c.c. centrifuge tube. The mixing is done by beating the material with a sterile glass rod or applicator. Then the tube is shaken frequently during a period of twenty minutes. The mixture is then centrifuged for ten minutes, thus leaving sputum and acid in contact for not more than thirty minutes. After decanting, the sediment is smeared in a thin layer over the surface of the slants.

The following two types of media are used, and a minimum of four tubes of each medium are seeded.

1. *A Modification of Sweeney's Medium*

Sterilized milk (see Sweeney's medium),	200 c.c.
Beef infusion broth (not alkalinized),	200 c.c.
Whole eggs,	800 c.c.
Glycerol,	60 c.c.
Aqueous solution of malachite green (2 per cent),	60 c.c.

These ingredients are mixed, the well beaten eggs being added last. The whole mixture is filtered through sterile gauze and tubed. The tubes, in slanting position, are coagulated

in the Arnold sterilizer for about half an hour, that is, just long enough to obtain the desired consistency

2 *Petragnani's*⁴⁵ Medium (1926)

Milk,	900 cc
Potato flour,	36 gm
Peptone,	6 gm
Potato (egg size pieces),	6

This mixture is kept in a boiling water bath with frequent stirring until it becomes sticky after this it is left in the water bath for from one to two hours. After cooling to 50° C, 24 whole eggs and 6 egg yolks, 70 cc of glycerol, and 60 cc of a 2 per cent aqueous solution of malachite green are added, the whole mixture is filtered through sterile gauze, tubed, and solidified in the same way as the preceding medium.

These media are kept in the incubator for not less than three days before use.

The majority of positive cultures will be obtained by the end of the second month; the earliest colonies may appear within ten days, but all negative tubes should be kept for no less than three months.

Colonies appearing on these media should always be examined in smear preparations, although I have invariably found that typical colonies are composed of typical acid-fast rods, I have been surprised to find repeatedly acid-fast rods in totally atypical colonies. Whether such apparently typical organisms with atypical colony formation are true tubercle bacilli is impossible to say at the present time. Some ten strains are being studied in regard to pathogenicity and tuberculin production. At all events it seems to be a good rule to examine all colonies regardless of their appearance before discarding the slants as "contaminated." Several authors have observed that scrapings from the surface of apparently sterile slants contain occasionally acid-fast rods, here again it is still undecided whether some or all or none of them are true tubercle bacilli.

c The Efficiency of Cultural Methods—It has been claimed by several writers that culture methods (usually a specific culture method) is fully as reliable as guinea pig inoculation. It is obvious, however, that so far no convincing proof has been adduced to substantiate such claims. All work, carefully controlled and on sufficiently large series of specimens (not on artificial mixtures of tubercle bacilli) would indicate that the actual number of positive results in guinea pigs is higher than on culture media, even though in a few instances cultures are obtained while a guinea pig inoculated with the same material fails to develop tuberculosis. For example, Stadnichenko and Sweany⁴⁶ found in a series of 200 specimens, 33 positive by animal inoculation and negative by culture, and 3 positive by culture and negative by animal inoculation.

Our own results may be mentioned in some detail, as follows. A series of 37 sputa from the Wm H Maybury Sanatorium in Northville was examined. All these sputa came from patients who had pulmonary tuberculosis and who had at some time had positive sputum. At the time of examination these sputa were negative on direct smear and had been so for at least one preceding examination. These 37 specimens were cultured and inoculated into guinea pigs.

In a second series of 55 specimens, including sputum, feces, exudates, tissues, urine, all negative on direct smear, the same type of study was performed. Table I summarizes the results.

TABLE I

A COMPARISON OF CULTURE AND GUINEA PIG INOCULATION IN 92 SPECIMENS FROM TUBERCULOUS PATIENTS*

NUMBER OF SPECIMENS	POSITIVE ON CULTURE (PEP CENT)	POSITIVE ON ANIMAL INOCULATION (PPF CENT)	CULTURE POSITIVE, GUINEA PIG NEGATIVE	GUINEA PIG POSITIVE, CULTURE NEGATIVE	POSITIVE BY ANY ONE METHOD (PPF CENT)
37	78.3	89.1	4	8	100.0
55	65.4	96.3	2	19	100.0
Total 92	70.6	93.5	6	27	100.0

*With the technical assistance of A. B. Mills and P. G. Kelly

On this series of 92 specimens it was determined how many culture tubes were positive, sterile, and contaminated. The results follow:

Total number of tubes	710
Number of positive tubes	338 or 47.6 per cent
Number of sterile tubes	354, or 49.9 per cent
Number of contaminated tubes	18 or 2.5 per cent

In the Desert Sanatorium in Tucson a total of 238 specimens (sputa, urine, exudates, tissues) was examined which were negative on direct smears. All of these were studied by concentration methods, cultures, and guinea pig inoculation. Table II shows the results.

TABLE II

A COMPARISON OF CULTURE AND GUINEA PIG INOCULATION IN 238 MISCELLANEOUS SPECIMENS*

NUMBER OF SPECIMENS (NEGATIVE ON DIRECT SMEAR)	POSITIVE AFTER CONCENTRATION	POSITIVE ON CULTURE	POSITIVE ON ANIMAL INOCULATION	POSITIVE BY ANY ONE METHOD	CULTURE POSITIVE, GUINEA PIG NEGATIVE	GUINEA PIG POSITIVE, CULTURE NEGATIVE
235	9	39	52	56	4	17

*With the technical assistance of J. P. Mote

In the 56 positive specimens in Table II, it was ascertained how soon a positive diagnosis could be made by cultural methods and by animal inoculation. Table III shows the percentage of positive diagnosis made at ten days' interval.

TABLE III

A COMPARISON OF CULTURE AND GUINEA PIG INOCULATION IN REGARD TO THE SPEED OF DIAGNOSIS. ACCUMULATIVE PERCENTAGES OF POSITIVE DIAGNOSIS OBTAINED IN 10 DAY PERIODS

DAYS	10	11-20	21-30	31-40	41-50	51-60
CULTURE	7.7	33.3	71.7	81.9	89.6	97.3
GUINEA PIG		0	30.7	63.4	86.5	94.3

Summarizing, the advantages of cultural methods are 1, independence of animals, 2, lower cost, 3, greater speed of diagnosis, and 4, freedom of interference by premature death of animals

On the other hand, the percentage of positive diagnoses is smaller than by animal inoculation. A complete sputum examination should include both cultural methods and animal inoculation

5 ANIMAL INOCULATION

For animal inoculation the sputum is prepared in exactly the same way as for culture. Any of the methods, antiformin, sodium hydroxide, or acids, may be used. The sediment must be neutralized in order to avoid necroses at the site of injection. The neutralized sediment is suspended in 1 to 2 cc of sterile saline solution. If possible, each specimen should be injected into two animals. The best way of injection is the subcutaneous route. Other types of injection, intraperitoneal, intrahepatic, intracerebral, which have been advised in order to hasten the development of tuberculosis, have definite disadvantages, namely, a greater early mortality from trauma and secondary infection, and the impossibility of watching the local lesion develop. The injection should be made in the region of the groin, because of the proximity of lymph nodes and because the infection should be started sufficiently distant from the middle line so that the first lymph node involvement is strictly unilateral.

A number of methods designed to hasten the development of tuberculosis have been given fair trials by a number of workers, including myself. All these methods seem not to attain their stated purpose to any appreciable extent.

It is frequently discussed whether guinea pigs used for inoculation should be tested with tuberculin before infection to ascertain that they are not spontaneously infected with tubercle bacilli. We believe this to be quite unnecessary for two good reasons. (1) notwithstanding a number of reports to the contrary, we believe that spontaneous tuberculosis in guinea pigs, kept under reasonably sanitary conditions of food and shelter, is extremely rare, (2) the autopic findings in a tuberculous guinea pig will establish beyond the possibilities of a doubt whether a spontaneous or an inoculation tuberculosis is present. It is probably one of the best established facts in experimental tuberculosis that at the site of first infection a local lesion develops and that the infection always spreads hence to the next draining lymph node. A guinea pig which shows tuberculous foci in the internal organs, but no primary lesion at the site of injection and in the regional lymph node, has acquired its tuberculosis not by injection, but by some other channel. It appears quite impossible that a spontaneous infection should enter by way of the subcutaneous tissue in the groin. Hence, a positive report of an animal inoculation should always specify that an *inoculation-tuberculosis* was found.

When should the autopsy be performed on an inoculated guinea pig? Starting about two weeks after inoculation, the animal should be examined at intervals for the appearance of the local lesion and the swelling of the deep inguinal lymph node. As soon as a local lesion or enlarged nodes are demonstrable, necrotic tissue from the inoculation ulcer, or an excised node may be examined in

smears stained for acid-fast bacilli. If they are found, the animal may be autopsied to confirm the preliminary diagnosis. If no acid-fast rods are seen, the animal should be kept alive for further observation.

Unless definite signs of tuberculosis (the local lesion¹) develop, no animal should be killed sooner than eight weeks after inoculation. No inoculated animal should be reported "negative" without complete autopsic examination. If one chooses one may perform tuberculin tests (intracutaneous) on inoculated animals starting about fifteen days after infection, this may help to arrive at an earlier diagnosis, but a positive tuberculin test without autopsy should never be accepted as final proof, nor should repeatedly negative tuberculin tests be accepted as full evidence for the absence of tuberculosis. Animals dying within the first three weeks after inoculation and showing no tuberculous lesions, should not be reported as negative. In such animals, tubercle bacilli or specific tissue changes may be found on microscopic examination.

6 THE CLINICAL SIGNIFICANCE OF POSITIVE AND NEGATIVE SPUTUM

The following remarks apply only to the results of thorough and complete examinations. By this is meant (1) a careful search for acid-fast rods in technically flawless smears, each search to last about ten minutes, if necessary, (2) frequent repetitions of such examinations, using, if necessary, sputum collected for several days, (3) the use of concentration methods, of cultural methods, and of animal inoculation.

Provided that these requirements be fulfilled both the finding of tubercle bacilli and the failure of finding them, constitute laboratory data of such high reliability as to be rivalled by few laboratory procedures. In a study from the Maybury Sanatorium, Pinner and Werner⁴ reported on sputum examinations on a consecutive series of 585 patients, of these 14 had nontuberculous pulmonary lesions, 33 had no pulmonary lesions, leaving a total of 538 patients with the clinical diagnosis of pulmonary tuberculosis. Of this group of patients, 36 had negative sputum in spite of exhaustive studies on their sputa. Clinically, these 36 negative sputum patients were classified as follows:

Apparently cured,	3
Apparently arrested,	20
Quiescent,	8
Active,	5

In a group of 507 patients with clinically active pulmonary tuberculosis, tubercle bacilli were demonstrated in 502, or a fraction more than 99 per cent. On the basis of such evidence, is it not a judicious claim that the failure to demonstrate tubercle bacilli in a patient's sputum should not be disregarded lightly as much of the routine teaching advises? Is it not reasonable to ascribe to such negative findings a considerable diagnostic or prognostic value? Should negative findings not be considered as indicating the likelihood of either a healed or healing tuberculous focus or of a nontuberculous pulmonary disease? Without trying to establish a hard and fast rule, we have found that the following is a practical working principle. If a patient has a demonstrable pulmonary lesion and

such symptoms as are consistent with that lesion, if, in addition, he has a sizeable amount of sputum (say 10 c.c. per day or more) then, the failure to find tubercle bacilli in his sputum should put the burden of proof on the shoulders of him who claims tuberculosis to be the etiologic factor. Such considerations become of special weight if the laboratory data are properly correlated with clinical findings. Although roentgenologic observations can, strictly speaking, never decide the etiology of a pulmonary lesion, the localization, upper or lower portions of the lung fields, gives indications which in conjunction with laboratory data enable the clinician at times to arrive at a definite diagnosis without strictly positive evidence.

What of a positive sputum? If we can be sure of the uncontaminated source of sputum excluding malingering with its not infrequent attempts at falsification in the matter of sputum, positive findings constitute a *prima facie* evidence of pulmonary tuberculosis. Possible errors are extremely rare, but when positive laboratory findings clash with negative clinical findings, two possible sources of error must be considered. 1. Acid-fast bacilli demonstrated in the sputum may be apathogenic organisms and not tubercle bacilli. A few such findings are reported in the literature. Pappenheim found large numbers of acid-fast bacilli, which he diagnosed as smegma bacilli, in a case of multiple pulmonary abscesses and bronchiectases. Similar findings are reported by Rabinowitsch,⁴⁶ who was able to cultivate these acid-fast apathogens. Since they grew in twenty-four hours to visible colonies, the differential diagnosis was easily established. Nonpathogenic acid-fast bacilli were demonstrated in the sputum in gangrene by Frankel,⁴⁷ in bronchitis fibrosa by Lichtenstein,⁴⁸ in bronchiectasis by Milchner.⁴⁹ Alexander¹ isolated acid-fast rods from the nasal secretion in ozena. Karlinsky²⁵ and Laabs³¹ from nasal secretions in normal persons, Moller²⁸ from saliva in normal persons. It must, of course, be remembered that the leprosy bacillus and certain elements, particularly spores of higher bacilli, are acid-fast.

From my own experience, I can quote but one instance in which nonpathogenic acid-fast rods were found in sputum. A young girl with a valvular heart lesion expectorated sputum twelve hours before death in which typical acid-fast rods were demonstrated on several smears. The autopsy, next day, did not reveal any sign of tuberculosis. A guinea pig injected with that sputum was killed more than ten weeks after the inoculation and was found to be grossly normal. But smears from the lymph node at the site of injection revealed numerous acid-fast rods.

The exact source of nonpathogenic acid-fast rods in human secretions is probably impossible to determine on account of the widespread occurrence of such organisms in dairy products, grass, manure, and water. The latter source must be kept in mind as a possible contamination during laboratory procedures. The cultural characteristic of some of the apathogens may be diagnostic, but a distinction by mere tinctorial and morphologic characteristics in smear preparations is unreliable.

To differentiate true tubercle bacilli from other members of the acid-fast group, it is best to use animal inoculation. If a specimen in which acid-fast organisms are demonstrated fails to infect a guinea pig, pure cultures should be

obtained of such doubtful acid-fast organisms and animal inoculations should be repeated with pure cultures

2 The possibility must be kept in mind that tubercle bacilli in sputum may be derived not only from a pulmonary focus but also from a tuberculous lesion in the upper respiratory tract. Such lesions in the absence of pulmonary tuberculosis are exceedingly rare. I saw a young woman from whose sputum a pure culture of tubercle bacilli was isolated. Clinically and roentgenologically, there was not the slightest sign of a pulmonary lesion. Her tonsils were removed and showed on histologic examination multiple caseated tubercles. In the absence of all evidence of a pulmonary lesion, with positive sputum findings and with or without symptoms suggestive of tuberculosis the possibility of a lesion in a hilar lymph node ruptured into a bronchus or the trachea must be considered. That such ruptured lesions may exist without producing parenchymal involvement, was shown by a case in which such a rupture, scarred at the time of autopsy existed and in which no trace of pulmonary tuberculosis barring an encapsulated primary focus was found.

These two sources of error are mentioned here not in order to point out their importance, but to emphasize their extreme rarity. On the other hand it should not be forgotten that Lowenstein² has mentioned repeatedly that he has seen a few cases in which tubercle bacilli from proved tuberculous lesions in man were apathogenic for guinea pigs.

The avian tubercle bacillus is known to infect man on rare occasions. In a recent review, Branch⁴ collected 15 cases from the literature, in only one of them were avian tubercle bacilli found in the sputum, the other 14 patients had exclusively or mainly extrapulmonary lesions. Avian tubercle bacilli are pathogenic for rabbits, producing on intravenous inoculation usually a septicemia, they are pathogenic for fowl, but in the usual dosage apathogenic for guinea pigs. The differentiation must be made on this basis of species pathogenicity.

The literature is replete with attempts to find prognostic indications in the number of bacilli and in their morphologic characteristics. First as to the number the bacilli, as mentioned are quite irregularly distributed throughout the sputum, a minute random sample can, therefore not be considered as representative of the entire specimen. Any attempt at a quantitative evaluation seems totally unjustified. The well-known Gaffky scale, with six or ten classes in regard to the number of bacilli claims a much finer distinction than is actually possible on the basis of the technique employed. A very rough classification, such as "very few," "moderate number," "very many" or simpler —, —, —, —, may have a limited justification. A continuous decline in the number of bacilli may be regarded as a possible favorable sign but occasional expectorations containing innumerable bacilli may be without prognostic significance, they are not rare occurrences in quiescent lesions.

If, for some reason, one wishes to obtain an accurate idea of the number of bacilli present actual counting must be done. Butschowitz⁷ published a procedure for this purpose.

As to the shape of bacilli long or short thick or thin solid or beaded I know of no convincing evidence that—as has been claimed—such variations in shape have any bearing on prognosis.

The number of diagnostic and prognostic laboratory tests for tuberculosis is appalling—specific and nonspecific serologic tests, blood chemical data, differential blood counts, ferment reactions of the serum, etc., they all have had their supporters. But, may it be stated emphatically in conclusion, that the clinical laboratory has no more important aid to offer to the clinician in practical work in pulmonary tuberculosis than thorough and complete bacteriologic studies of the sputum.

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THE ISOLATION AND IDENTIFICATION OF PATHOGENIC BACTERIA IN FECES*

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THE detection of pathogenic bacteria in feces is one of the most complicated bacteriologic problems which a diagnostic laboratory encounters. Their isolation, in the first place, is attended by many technical pitfalls, and any significant organisms which may be found require intelligent and careful work for their identification. There are many intestinal bacteria which closely resemble the known pathogens but which so far have no definitely ascribed rôle. Their significance is uncertain and their presence is often confusing. Hence one can hardly be too careful in the procedures used for purposes of identification. In the majority of instances, of course such as typhoid fever, the offending organism is readily identified by comparatively simple cultural and agglutinative reactions. But when one approaches the paratyphoid group and, particularly the dysenteries, skill and experience are needed. It is here, in this group of diseases, due to their comparatively infrequent occurrence and their vague clinical symptoms, that the laboratory is called on to actually establish the diagnosis. Thus, even though such examples appear comparatively infrequently, it is, nevertheless, essential that the laboratory be prepared to make an intelligent examination. A successful attack on one obscure case of this nature may enhance the prestige and increase the reputation of a laboratory more than any number of ordinary, routine examinations.

The subject matter of this paper makes little pretense of presenting new material. Its chief purposes are (1) to bring together in convenient form methods which have been found useful and practicable for the bacteriologic examination of fecal specimens, and (2) to discuss the significance of some of the pathogens which leave the body through the intestinal tract. We make, in our Central Laboratory, of necessity, large numbers of such examinations and have had thrust upon us the evolving of methods which can readily be utilized in our branch laboratories. The practices adopted must be neither too expensive nor too time-consuming for a laboratory with limited personnel and funds and, at the same time, must give assurance of thoroughness and accuracy. This has necessitated the sifting of known methods—which are legion—and in some instances, the development of new procedures.

One is impressed, in studying the available treatises of clinical diagnostic methods, by the casual manner in which the bacteriology of the feces has been handled. In fact, the following surprising statement may be found,¹ "For laboratory diagnosis (of typhoid fever) blood cultures during the first week and agglutination tests during the second week and onward are the practical methods." Probably such hasty dismissal of bacteriologic methods is due in large part to their very multiplicity. There is, perhaps, no other diagnostic procedure involving bacteriologic methods which has received less standardization.

*From the Laboratories of Alabama State Board of Health, Montgomery.

than that of the intestinal tract. Almost every bacteriologist has his favorite procedures which seem to be successful in his own hands, consequently, he hesitates to accept new methods or modifications of old ones. The situation is comparable to that formerly existing in the serologic diagnosis of syphilis. One may hope therefore that eventually, there may be established for the diagnosis of intestinal pathogens criteria which will serve a similar useful purpose in clarifying known facts and crystallizing the technical chaos.

In view of the accumulating evidence of the lack of diagnostic value of agglutination tests with the patient's serum the extent to which the clinician still relies upon these simple procedures is regrettable. The only certain means of establishing the nature of an infection is the isolation of the causative organism. This is particularly true of the typhoid-paratyphoid-dysentery groups, where the phenomenon of cross agglutination occurs so frequently, to say nothing of the development of agglutinins as a result of vaccination and missed or subclinical infections. It is the duty of the diagnostic laboratory to educate the clinician in the proper use of the laboratory, encouragement in the routine use of cultural methods must presuppose adequate equipment mental as well as physical, on the part of the laboratory itself.

From our standpoint the important bacteria of the intestinal tract are *B. typhosus*, the *Salmonella* group and the dysenteries. The first problem in the bacteriologic examination of any intestinal infection is assurance of a dependable specimen with which to work. Even more than with other laboratory examinations a successful result is governed largely by the intelligence and care used in the collection of the specimen, together with the promptness and skill attending the initial steps in the examination. Unless the feces can be immediately taken to the laboratory and cultures made without delay, as in a hospital, special methods for preservation are necessary. Even under ideal conditions, some means of preserving the specimen for further study are desirable since, as will appear later, repeated platings materially increase the percentage of positive results.

For these reasons a large amount of study has been focused on the treatment of the specimen prior to the bacteriologic examination. The chief effort, of course, is to prevent or retard the enormous overgrowth of *B. coli*, which occurs so rapidly in untreated feces.² Most of the methods depend upon the use of brilliant green. In the description of the technic which follows, we have utilized the sharp selective action of this dye for the preservation of the specimen itself, rather than the customary usage in preliminary enrichment cultures. Since the dye has no selective action on the dysentery bacilli, other methods must be used for their isolation. This is one of the chief reasons for the difficulty attending the isolation of members of this group.

PREPARATION OF MEDIA USED IN ISOLATION AND IDENTIFICATION

In this description of methods of isolation and identification we have included details only of those procedures not readily found in most textbooks of bacteriology. We have not given the formulas for example for plain broth and Russell's double sugar agar because they are readily available elsewhere, but, on the other hand we have included the details of such media as Simmons'

citrate agar and Jordan's tannate medium, not only because they are comparatively new, but also because their usefulness in the differentiation of intestinal bacteria warrants wider application in routine diagnostic practice.

Repeated plating of the specimen is another procedure, the importance of which should be stressed. It not infrequently happens that a positive result is obtained only on the second or even the third day plates, after further diminution in the total bacterial flora has occurred, and, as in the case of brilliant green bile cultures, an actual increase in the numbers of typhoid or paratyphoid colonies has taken place.

Brilliant Green Solution—The brilliant green at present obtainable is much less satisfactory for the isolation of typhoid bacilli from feces than that formerly obtainable, owing to a decrease in its selective action. While batches of the dye can be found that are fairly suitable for brilliant green agar, it has been impossible to obtain any recent dye that will serve the purpose in bile. For this reason attempts were made to alter or modify the dye in the hope that its selective action could thereby be increased.

The following treatment has been found, in most instances, to increase greatly the selective action of brilliant green. A 5 per cent solution of the dye in N/3 hydrochloric acid is evaporated to a syrupy consistency* over a boiling water bath. The evaporated dye is redissolved in distilled water to make a 5 per cent solution and kept for use. When the dye is dissolved in the acid, a color change takes place—a yellow or burnt orange—but the final color is again green.

The proper concentration of the treated dye is determined by titration. Fifteen c.c. of bile (Digestive Ferments Co., dehydrated bile) is placed in each of a series of 1 oz. bottles. To the first bottle is added 2.5 c.c. of the 5 per cent stock solution (concentration 1:120), to the second, 2.0 c.c. (1:150), and decreasing amounts to the succeeding bottles to a concentration of 1:300 or 1:400, with intervals between the concentrations of not more than 20 to 25 per cent. In our experience the greatest selective action falls within this range, usually around 1:200. Several (4-6) sets covering this range should be prepared to permit testing with several specimens of feces. The bottles are labelled and sterilized in the autoclave.

To each bottle is then added 1 c.c. of a 1:10 suspension of feces in salt solution and 0.1 c.c. of a twenty-four hour broth culture of *B. typhosus*. The bottles are kept at room temperature and a loopful from each streaked on brilliant green agar plates at the end of each twenty-four hour interval for three days. That concentration of brilliant green is used, for the particular batch of bile in question, which gives the greatest yield of typhoid colonies, together with the most pronounced inhibition of fecal bacteria.

This titration is absolutely essential to the success of the method. It is advisable, also, as a further check, to test the finished batch of brilliant green bile with several different specimens of feces seeded with typhoid. This medium is satisfactory for blood and urine cultures, as well as for feces.

The brilliant green agar used for plating must also be carefully titrated. We prefer brilliant green Endo agar, as it gives, in our hands, better differentiation between lactose and nonlactose fermenting colonies. Whether Krumwiede's agar is used or brilliant green Endo agar, the proper concentration of dye is determined for each lot of agar.

It cannot be emphasized too strongly that the success of the method lies in carefully determining the proper concentration of the dye for each new batch of brilliant green, each new batch of bile, and each new batch of agar. It is possible with this method to obtain, with many specimens of feces, almost pure cultures of typhoid for several days after the preparation of the specimen.

*The end-point described for the evaporation process seems somewhat indefinite but as a matter of fact there is a fairly wide range within which the action of the dye is satisfactory. If evaporation is stopped before the syrupy consistency is reached the resulting solution is inhibitory to *B. typhosus* while if carried to a gummy consistency it is no longer inhibitory to *B. coli*. Between these points, batches of dye with reasonably good selective action can almost always be obtained.

†Comparative studies at 20° C. and 37° C. indicate that the selective action of the dye is much less pronounced at the higher temperature with consequent increase in numbers of *B. coli*.

Preparation of Bile Medium—Dissolve 100 grams Bacto oxgall (Digestive Ferments Co.) in 1000 cc distilled water, and sterilize for thirty minutes at 15 pounds pressure. The Digestive Ferments Co. labels bear a number. All bile of the same number may be used without additional titrations for the proper brilliant green content.

Preparation of Brilliant Green Endo Agar—Forty-one and five-tenths grams of dehydrated Endo agar (Digestive Ferments Co.) are dissolved in 1000 cc of water and sterilized for fifteen minutes at 15 pounds pressure. The medium is then given a lot number, titrated and placed in the cold room until ready for use. Each reference number on the label of the bottle of dehydrated agar is noted and each new lot is numbered.

Titration—Five flasks, each containing 200 cc of Endo medium, are melted, and to these are added increasing concentrations of the 5 per cent brilliant green solution covering the range within which the proper dilution lies (this is usually between 1:100,000 and 1:250,000).

A plate of each dilution is then streaked for two successive days with a loopful from a brilliant green bile culture prepared twenty-four hours before by adding 0.1 cc of a twenty-four-hour broth culture of *B. typhosus* and 1.0 cc of 1:10 dilution of feces in salt solution to 15 cc of brilliant green bile. That concentration is chosen which gives the most complete inhibition of fecal bacteria, and at the same time no marked inhibition of *B. typhosus* as evidenced by the number and size of the colonies.

It is advisable to set up three or more such bottles using a different specimen of feces in each, since some feces contain organisms not readily inhibited by the dye and others may yield almost pure cultures of *B. typhosus*, in either case, consequently, a clear-cut titration is not obtained.

Preparation of Sugar Broth—Prepare plain beef extract broth, to which is added 1 per cent Andrade indicator (reaction adjusted to indicator— P_H 7.2-7.4). Place in small tubes ($\frac{1}{2}$ " by 4") in 2 cc amounts with fermentation tubes* and sterilize for thirty minutes at 15 pounds pressure. Then add aseptically the sugar solutions (0.05 cc of 20 per cent solution, to make 0.5 per cent). Mark sugars by coloring cotton plugs with different dyes as a means of identification and sterilize in Arnold sterilizer for sixty minutes. Incubate for sterility and test with known stock cultures.

Stock Sugar Solutions—Weigh accurately 1 gram of the sugar and dissolve in 5 cc of distilled water (making a 20 per cent solution). Sterilize for twenty minutes at 15 pounds pressure.

SODIUM POTASSIUM TARTRATE MEDIUM¹

Agar	20 grams
Distilled water	1000 cc
Alcohol sol. phenol red (2 per cent)	12 cc
Difco peptone	10 grams
Sodium potassium tartrate	10 grams
Sodium chloride	5 grams

*These are conveniently made by sealing 6 to 8 mm tubing at the end and cutting off in 10 to 12 mm lengths.

Adjust medium to P_H 7.678 tube, sterilize for twenty minutes at 15 pounds pressure and slant. Incubate for sterility and test with known cultures. Store in the cold room. It has been our observation that the medium should be fresh. Tubes which have been made for some time (two weeks or longer) even though stored in the ice box fail to give sharp reactions.

SIMMONS' CITRATE AGAR

Agar	20.0	grams
NaCl	5.0	grams
MgSO ₄	0.2	grams
(NH ₄) H PO ₄	1.0	grams
K-H PO ₄	1.0	grams
Sodium citrate (anhydrous)	2.0	grams
or (5½ H ₂ O)	2.77	grams
Distilled water	1000	cc
Bromthymol blue (1.5 per cent in 95 per cent alcohol)	10	cc

Adjust to P_H 6.8, add indicator tube sterilize for twenty minutes at 15 pounds pressure, and slant. Incubate over night for sterility, test with known cultures and place in cold room.

Collection of the Specimen—The brilliant green bile is used for blood (5 to 10 cc), urine (10 to 15 cc) and feces (about 0.1 gram or about the size of a small bean). It is very important to give instructions that only a small amount of feces be added as larger amounts will absorb so much of the dye that overgrowth by fecal bacteria results.

Isolation and Identification—For the typhoid-paratyphoid group, specimens are received into the laboratory in brilliant green bile. For the dysenteries the usual 30 per cent glycerin in 0.85 per cent NaCl is employed. Usually, upwards of twenty-four hours elapse after collection before the specimen is examined. The specimens thus preserved, are streaked on three succeeding days on brilliant green Endo agar and on plain Endo plates, one loopful from the surface of each bottle being used, without agitation of the contents, the bottles being left at room temperature in the meantime. The purpose of the three successive platings is to permit multiplication in the bile, of original small numbers of organisms to a point where they may be detected on a plate streaked with one loopful of the culture, and to permit further progressive decrease in the numbers of *B. coli* and other fecal bacteria which interfere.

A macroscopic slide agglutination test is made from characteristic colonies on the plates. This gives preliminary evidence of the nature of the organism. If the colony is typical and if the performance of the particular serum and the dilution in which it is used are known by constant daily experience, a preliminary report of specimens from clinical cases may be immediately made on the basis of this test alone.* If conservatively used, it is invariably confirmed by the subsequent fermentation and agglutination tests.

*The test is performed by placing a loopful of the appropriate serum dilution on the slide. Touch the point of the needle into the colony to be tested and emulsify in the loopful of serum. A positive test is indicated by the formation of large clumps plainly visible to the naked eye. Each serum used for the macroscopic slide agglutination test must be titrated against a number of closely related organisms (e.g. *B. typhosus*, *paratyphosus* A and B, *B. morrisoni*) and the lowest dilution used which gives definite rapid agglutination (in from ten to fifteen seconds) with its homologous strain and no cross agglutination with the closely related strains. (See Park and Williams, *Pathogenic Microorganisms*, Philadelphia, ed. 8, p. 204.) The clumps form usually almost at once. If agglutination is delayed or is incomplete it should be interpreted with caution.

Regardless of the results of the slide agglutination suspicious colonies are transferred to Russell double sugar tubes. From the Russell tubes fermentation tests are made on the following carbohydrates: adonite, mannite, rhamnose, saccharose and sorbite. These five fermentable substances have been found particularly useful for purposes of identification. Adonite is perhaps the least essential but it often serves to detect certain members of the colon group which yield colorless colonies on Endo after twenty-four hours' incubation.

In addition to the carbohydrate media tubes of citrate agar, tartrate agar and peptone water (for indol test) are inoculated.

At the same time a plain agar slant is inoculated for a macroscopic tube agglutination test and the culture (Russell tube) from which the sugar broths are inoculated is emulsified in peptone water and a loopful from this streaked on Endo plates. This last step gives valuable information regarding the characteristics of the colonies in pure culture and constitute a check upon the purity of the culture in the carbohydrate media.

We have found this "re-streak" plate particularly useful as an aid in classification. Besides the colony characteristics and the assurance of a pure culture

TABLE I
CULTURAL REACTIONS OF NONLACTOSE FERMENTING INTESTINAL BACTÆRIA

CULTURE	RUSSELL DOUBLE SUGAR AGAR	CARBOHYDRATE REACTIONS					INDOL	CITRATE	TARTRATE
		ADONITE	MANNITE	RHAMNOSE	SACCHAROSE	SORBITE			
<i>B. typhosus</i>	-	-	-	-	-	-	-	-	A
<i>B. paratyphosus</i> A	⊖	-	⊖	-	-	⊖	-	-	-
<i>B. paratyphosus</i> B (Schottmüller)	⊖	-	⊖	⊖	-	⊖	-	-	-
<i>B. paratyphosus</i> B (Aertgeke)	⊖	-	⊖	⊖	-	⊖	-	-	A
<i>B. morgani</i>	- or ⊖	-	-	-	- or ⊖ S	-	-	-	AA
<i>B. enteritidis</i>	⊖	-	⊖	⊖	-	⊖	-	-	A
<i>B. supestrifer</i> (<i>S. cholerae</i> suis)	⊖	-	⊖	⊖	-	⊖	-	-	A
<i>B. dysenteriae</i> (Shiga)	-	-	-	-	-	-	-	-	AA
<i>B. dysenteriae</i> (Flexner)	-	-	-	-	-	-	-	-	A
<i>B. dysenteriae</i> (Strong)	-	-	-	-	-	-	-	-	A
<i>B. dysenteriae</i> (Y)	-	-	-	-	-	-	-	-	A
<i>B. dysenteriae</i> (Sonne)*	-	-	-	-	- S	-	-	-	A
<i>B. proteus</i>	⊖	-	-	-	⊖	-	-	-	AA

- No acid or gas - acid ⊖ acid and gas A acid butt AA acid butt acid slant
S slow fermentation (7-10 days) * ferments lactose slowly

for further study, this second plating from the Russell tubes frequently gives clear evidence of slow lactose-fermenting strains. Although the colonies on the original plate may have been colorless and the reaction on Russell medium typical of a *Salmonella* organism, when plated again on Endo, such cultures often ferment lactose rapidly. There is a consequent saving of time in further identification which might have been attempted, had this single step been omitted.

If a strain is isolated which gives typical cultural reactions but which does not agglutinate with the homologous monovalent serum indicated by the carbohydrate reactions, it is subcultured daily on plain agar slants. Usually six or eight transfers are sufficient to render it agglutinable. Strains isolated from the feces are, in our experience, not often magglutinable. Blood cultures, on the other hand, frequently show magglutinable colonies on the plate (as evidenced by slide agglutination). The great majority of these become agglutinable as soon as they have been transferred to Russell tubes.

INTERPRETATION OF RESULTS

In the foregoing description of methods of identification too much significance should not be attached to minor variations. For example, Morgan's bacillus may ferment saccharose slowly.⁶ A number of strains in our collection have been found to possess this characteristic. It is, of course, well known that freshly isolated cultures of the dysentery group may give atypical reactions. Agglutination with known monovalent serums must always be the final test, particularly after several transfers on artificial media. The serological characteristics, as well as the cultural, tend to become stable, as a rule, after a few subcultures.

A useful lead to the significance of any unusual organism isolated from the feces may be gained from an agglutination test with the patient's serum. This is particularly true of the paratypheries and certain of the *Salmonellas* which are not only difficult to classify without extended study, but are known to occur sometimes in the healthy intestine. Tests with the patient's serum have been particularly useful, in our own experience, in determining the significance of Morgan's bacillus. This organism has been reported⁷ as the cause of clinical paratyphoid fever, but since it is not infrequently found in normal stools, its isolation alone is not sufficient to establish it as the cause of the infection. In a series of cases which we have studied,⁸ we found that agglutinins were invariably produced against the homologous culture and that the agglutinin titer rose during the course of the infection. That an organism can be shown to have caused a reaction on the part of the host, and, further, that this reaction is an increasing one, as evidenced by a subsequent stronger agglutination, constitutes strong presumptive evidence that it is the cause of the clinical manifestations.

SPECIFIC CONSIDERATIONS

Typhoid.—The isolation of *B. typhosus* from the feces is sufficient, coupled with typical clinical symptoms, to establish the diagnosis. With atypical clinical

cal symptoms a positive finding in the feces must be qualified by the possibility of the carrier condition

One of the chief objections to cultural methods, on the part of clinicians, is the delay in obtaining the result. A preliminary report, based on colony agglutination, may often be given within twenty-four hours. If the colony is typical* in appearance and specific agglutination occurs, a preliminary report is justified, with no more delay than with a simple Widal test. It cannot be too strongly emphasized that experience is required for the colony agglutination. The chief pitfall is nonspecific agglutination, due to the use of too low a dilution of serum within the range of group agglutination. Some typhoid serums contain these group agglutinins to a greater degree than others, and are, therefore, not so useful for this purpose. All preliminary reports based on colony agglutination should be confirmed by subculture on Russell's double sugar agar, from which the usual confirmatory tests may be carried on.

Our experience with feces cultures for the diagnosis of typhoid has been very satisfactory, with the result that our laboratory now receives more specimens for cultural methods than for agglutination tests, thus indicating that the practitioner will utilize any laboratory procedure that demonstrates results.

The typhoid bacillus is present in the feces from the time of onset of the disease, and in some cases even earlier (precocious carriers).⁹ Feces culture, therefore, is a useful diagnostic method, regardless of the stage of the disease. In 108 cases of clinical typhoid fever from which a single specimen of feces was submitted, varying between the third and the forty-eighth day of the attack, we isolated the typhoid bacillus in 86 or 80 per cent.¹⁰ Of those received during the first week, 66 per cent were positive, during the second week, 77 per cent, during the third week, 79 per cent, and after the twenty-first day, 100 per cent of this series yielded positive results.

Paratyphosus A—Judging from the literature this is relatively rare. Little difficulty is usually encountered in its identification.

Paratyphosus B—It now seems clear that this term has been applied to two organisms which are quite distinct, bacteriologically and clinically. Jordan's classification into *Salmonella schottmulleri*, which usually causes prolonged paratyphoid fever, and *Salmonella aertryckeri*, associated with explosive food poisoning, has cleared up a great deal of the existing bewilderment. While this differentiation is not without exception,[†] the two strains are bacteriologically distinct and should not be confused. Heretofore, many bacteriologists, particularly in Germany, have failed to make this distinction, and there is still a tendency there to consider the *aertrycke* type merely a variety of the species *paratyphosus B*. The English workers, on the other hand, have complicated the situation by describing many different strains—"Mutton," "Newport," etc.—which are, apparently, practically identical with *S. aertryckeri*.¹¹ That this confusion is becoming more confounded is indicated by a recent description:¹²

*It is interesting to note that the classical textbook description of the appearance of the colony of *B. typhosus* is that of the rough variant. It has been our experience that this form rarely occurs in freshly isolated cultures; the smooth form predominates with a regular edge and smooth glistening surface. (See Grinnell F. B. J. Immunol. 19: 457, 1930.)
[†]We have isolated the Schottmüller type from two explosive outbreaks of food poisoning: one due to sausage and the other ground beef sandwiches.

of an organism seemingly belonging to the *aertrycke* species, which, because it shows certain serologic differences has been given the name "Salmonella Eastbourne." It is questionable whether minor antigenic differences warrant the creation of a new species. On this basis it would be just as logical to name each individual strain of *S. morganii* as a separate species. As Jordan remarks¹¹ "Until the range and significance of antigenic variation become more fully understood than they are at present the multiplication of specific names may well be kept on a conservative basis."

Paratyphosus "C"—It is now generally believed that this strain found in the Balkans and elsewhere during the war, is identical with *B. supestifer* (*S. cholerae suis*)

S. Morganii No 1—Similar to the paratyphoid bacilli in its greater resistance to brilliant green this organism is more easily isolated from feces than is *B. typhosus*. As already mentioned,^{7, 8} it has been recovered from blood cultures as well as from the feces in cases clinically resembling paratyphoid fever. It has also been reported¹² as the cause of a severe ulcerative colitis. Since it is found, not infrequently, in the feces of normal persons, its significance is doubtful unless the isolation from the intestinal tract is supported by a positive blood culture or a positive agglutination test with the patient's serum. At any rate, such a finding should not be summarily dismissed as of no importance.

S. Enteritidis—This organism, together with *S. cholerae suis*, has been found associated with cases of food poisoning but they are rarely isolated from human feces. In the study of some 20,000 feces cultures in Alabama we have not once found these bacteria.

The Dysentery Group—Here, more than with any other intestinal pathogen, is the laboratory confronted with technical difficulties on the one hand, and, on the other, with the necessity of isolating the causative organism as the only sure proof of the cause of the infection. Even more common than for *B. typhosus* is the occurrence of dysentery agglutinins in normal persons.¹⁴ Cross-agglutination between the different members of the group is a common phenomenon and one must resort to cultural methods to establish the diagnosis. Yet the procedures at our command are notoriously uncertain. Enrichment methods fail because there is no selective medium as in the case of the typhoid-paratyphoid group. The dysentery bacilli are fragile, and are overgrown rapidly, necessitating the use of fresh specimens.

The ideal procedure for collection of the specimen is to hospitalize the patient, collect the specimen with the aid of a proctoscope, and start the cultures at once, at the bedside if the laboratory is at all remote. The specimen should be carefully examined for flecks of blood and mucus. These should be selected, rinsed in broth or salt solution, and streaked on Endo plates. Lacking these facilities, fair results may be obtained by preserving the feces in 30 per cent glycerin.

The most common member of this group in the United States is the Flexner strain, but in outbreaks of dysentery, variants either cultural or serologic, may occur¹⁵ and a strain should not be too hastily discarded as of no importance, merely because it does not conform in all respects to the type. Here again,

agglutination tests with the patient's serum may be useful in establishing the significance of a questionable culture. According to Koser and his associates,¹⁶ the Sonne type is not uncommon as a cause of dysentery in this country.

Related Bacteria of Doubtful Significance—Everyone who has had extended experience with fecal bacteriology has found numerous unclassifiable bacteria which sufficiently resemble the established pathogenic types to cause at least momentary confusion.

Since the first differentiating criterion upon which all differential media are based is failure to ferment lactose naturally any nonlactose-fermenting colony found on the plates should rightly excite suspicion. Too much importance should not be placed on the colony characteristics such as shape, size, opacity, etc. since colonies of the same strain may vary markedly, particularly if the organism is susceptible to dissociative changes. The culture on Russell's double sugar agar furnishes a basis for the rapid elimination of many of these bacteria which have no significance as disease-producers. In most instances the character of the growth may at once eliminate the culture as of no importance, without the necessity for further study. Slide agglutination is a useful rapid method of obtaining preliminary information. The sugar reactions followed by specific agglutination in known monovalent serum then establish the characteristics necessary to indicate whether or not further study is warranted.

In spite of these procedures which serve, in most instances, either to identify or eliminate the culture, one not infrequently finds strains which differ from some classified member of the *Salmonella* group only in their action on a single carbohydrate. We have a collection of some 200 such strains, almost every one differing in some respect from the others. They have been isolated from normal feces as well as from patients with enteric disease. Some resemble paratyphus B except in their inability to utilize sorbite. Others fail to ferment rhamnose and none agglutinates in any paratyphoid B serum which we have used. From time to time, considerable study has been given to these cultures, but their chief bacteriologic importance seems to lie in their confusing resemblance to the known pathogenic *Salmonellas* with the consequent necessity for their differentiation in arriving at the etiologic agent in any given case.

CONCLUSIONS

We have tried to show (1) that intestinal bacteriology has an essential place in the diagnostic laboratory and (2) that bacteriologic studies of the feces are of practical importance, from the standpoint of both promptness and accuracy.

Much excellent clinical material which would add to our knowledge of this difficult field of bacteriology undoubtedly goes to waste for lack of opportunity for thorough study. If more of these patients could be made available for bacteriologic examination not only would the laboratory benefit by the accumulation of information but clinical medicine would be improved by more accurate diagnosis in many baffling and obscure conditions.

The methods at our command are by no means perfect, but it has been our experience that, when intelligently and conscientiously used, they compare

favorably with many of our accepted examinations. Their very lack of perfection and our inadequate knowledge are incentives to more extensive, as well as intensive, study. There is a large field for research among the intestinal pathogens, not only in technical methods for their isolation and identification, but also to increase our knowledge of their significance as the causative factors in enteric diseases.

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EXAMINATION AND IDENTIFICATION OF PROTOZOA*

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INTRODUCTION

THE protozoa found in human tissues and exudates include forms belonging to all four major groups of the Phylum Protozoa, namely the Rhizopoda, as illustrated by *Endameba histolytica*, the Mastigophora as illustrated by the intestinal flagellates and the trypanosomes, the Sporozoa, as illustrated by the malaria plasmodia, and the Ciliata, as illustrated by *Balantidium coli*

Some of these organisms are strictly tissue parasites, others may be regarded as facultative pathogens, and still others are harmless forms of a coprozoic nature living in or passing through the intestinal tract For purposes of convenience it is desirable to consider these protozoa under two categories, (1) those living in the intestinal tract and adjacent organs and (2) those primarily involving the hematopoietic organs blood stream and other body tissues

I PROTOZOA OF THE INTESTINAL TRACT AND ADNEXA

1 *The amebae (Class Rhizopoda, Order Amoebida)*

The amebae found in the gastrointestinal tract of man include *Endameba histolytica*, *Endameba coli*, *Endameba gingivalis*, *Endolimax nana*, *Iodameoba butschlii* (vel williamsi), *Dientamoeba fragilis* and the rare form *Caudamoeba sinensis* One of these species (*E. gingivalis*) is parasitic in the human mouth, the others live in the lower levels of the intestine, being confined almost exclusively to the large bowel Of these latter *E. histolytica* and *Caudamoeba sinensis* are tissue parasites, while the remaining species live free in the lumen of the large bowel and are regarded as nonpathogenic

TECHNIC FOR EXAMINING INTESTINAL EXUDATES FOR PROTOZOA

In routine laboratory diagnosis I use the following equipment

- 1 A good compound microscope, with periplanatic oculars $\times 6$ and $\times 10$, 16 mm 4 mm and 19 mm objectives (either achromatic or fluorite), and a mechanical stage
- 2 A daylight blue electric lamp, used as a constant source of light
- 3 Fecal slides (40 \times 75 mm), of clear white glass, clean of dirt and oil film
- 4 Cover glasses (22 mm square, not over 18 μ in thickness), spotlessly clean
- 5 Toothpicks and specimen applicators
- 6 A dropping bottle of physiologic saline solution
- 7 A dropping bottle of Donaldson's iodine solution (saturated solution of iodine in 5 per cent aqueous potassium iodide)

*From the Parasitology Laboratory Department of Tropical Medicine Tulane University Medical School

The following technique is employed. A fleck of the specimen to be examined is thoroughly mixed on the slide in a drop of physiologic saline solution. This is streaked across two cover glass widths and a cover glass placed over one half of the film. A small drop of the iodine solution is then mixed with the uncovered portion of the film and covered with a second cover glass. The film is first examined with the 16 mm objective and any suspicious objects then studied with the 4 mm lens. On the unstained side active stages (trichozoites) and cysts will be found in their natural hyaline color, on the iodine stained side these organisms will be stained so that the chromatin material stands out in light relief against the yellowish brown cytoplasm. In other words, the picture with the iodine staining is essentially the reverse of that obtained by the iron hematoxylin technique. The iodine also stains the glycogen masses a more or less deep mahogany brown.

A 2 gram portion of each formed or semiformed specimen is also diluted with 20 c.c. of water, strained through gauze and centrifuged. This frequently yields cysts where the undiluted film is negative. At least three separate specimens and preferably six are requested for examination before a diagnosis of "negative" is finally entered. Freshly passed specimens (not over thirty minutes old) are most satisfactory. Specimens containing oil of any kind are practically worthless for examination and one passed after a saline purgative is almost as unsatisfactory.

Occasionally (perhaps in 5 per cent of the cases), it is desirable to check the temporary iodine films with a Schaudinn-fixed non-hematoxylin stained preparation. But experience with over 50,000 fecal examinations has shown that the one method is as dependable as the other, and the simplicity of the former leaves much to be said in its favor. However, in case hematoxylin stained preparations are desired, the following technique is recommended.

- 1 Smear fecal material on slide evenly and not too thick
- 2 Place slide in Schaudinn's fluid¹ heated to a temperature of 60° C
- Leave in two minutes
- 3 Transfer to 70 per cent alcohol, two minutes
- 4 Transfer to 70 per cent iodine alcohol, two minutes
- 5 Transfer to 70 per cent alcohol, two minutes
- 6 Transfer to 50 per cent alcohol, two minutes
- 7 Wash in running water two minutes
- 8 Transfer to 2 per cent aqueous non alum solution at 40° C, two minutes
- 9 Wash in running water three minutes
- 10 Transfer to 1/2 per cent aqueous hematoxylin two minutes (This time may vary according to the strength of the stain)
- 11 Wash in water two minutes
- 12 Destain in cold 2 per cent aqueous non alum
- 13 Wash in running water ten to fifteen minutes
- 14 Run through 50 per cent, 70 per cent, 80 per cent, 90 per cent, and 100 per cent alcohol, two minutes each

¹Schaudinn's fixing fluid. Prepare the solution as follows. Saturated solution of mercuric chloride in distilled water 200 c.c. 95 per cent alcohol 100 c.c. glacial acetic acid, 10 c.c. (The acid should not be added until the fluid is to be used.)

15 Xylol two minutes

16 Mount in balsam or euparal, using a No. 1 cover glass

ENDAMEBA HISTOLYTICA (Fig. 1 A-F)

This organism is the well-known tissue pathogen. It commonly lives in the wall of the large bowel, particularly the cecum and appendix, but occasionally the adjacent levels of the ileum may be involved. Secondly, this ameba may migrate through the blood stream to the liver, where a focus of infection may develop, more rarely the lungs, brain, and other organs may be parasitized.

In an acute or subacute enteritis, the organism will be passed in the active (trophozoite) stage (Fig. 1 A) in a liquid or semiliquid exudate containing many red blood cells, necrotic tissue cells, but relatively few pus cells. In order

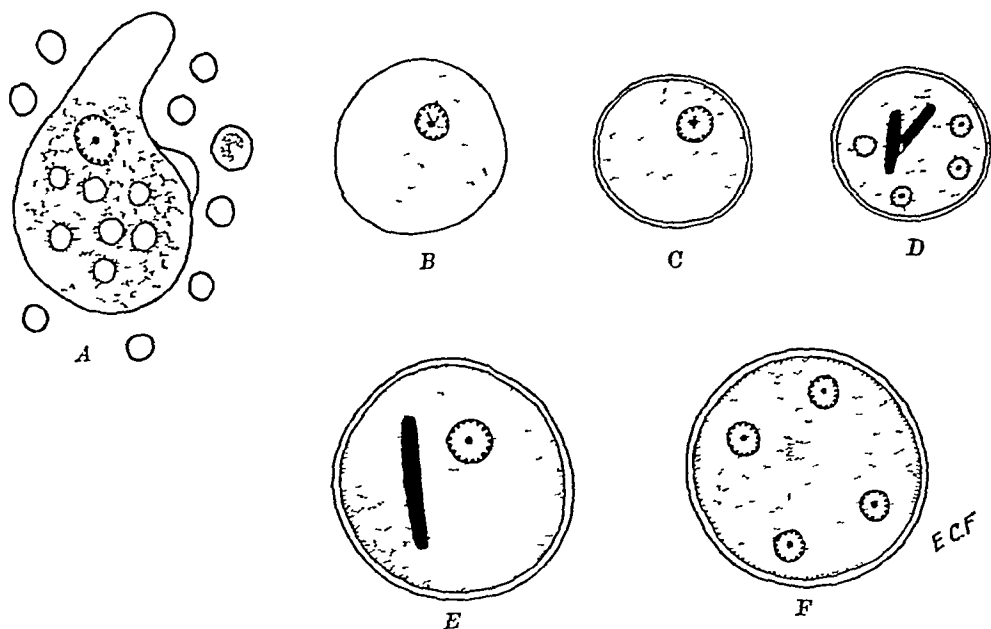


Fig. 1—*Endameba histolytica*. A, active trophozoite. B, precystic stage. C-F, small and large races of cysts. A, B, $\times 1300$; C-F, $\times 2000$.

to make a satisfactory diagnosis of such material it is essential that the freshly passed exudate should be examined immediately upon being passed, care being taken that it is not chilled. A fleck of the bloody mucus is placed on a clean microscopic slide, diluted with tepid physiologic saline and mounted with a clean cover glass. These amebae will be seen moving about quite actively in the medium, throwing out their clear pseudopodial processes first in one direction then in another, with the nucleus near the forward end of the organism. They vary in size from 18 to 40 μ . Frequently they will be found to contain ingested red blood cells and rarely bacteria. While it is at times possible to find this active stage of *E. histolytica* in the stool for several hours after evacuation, it frequently dies in a half hour or less, particularly in an exudate where putrefaction is going on. In a semidegenerate condition, it may be confused with monocytes, which, however, have much larger nuclei. The large mono-

nucleus in bacillary dysentery exudate are frequently mistaken for *E. histolytica*. It is a safe principle never to diagnose such a form as an ameba unless definite movement is demonstrated. At times these amebae have degenerated in the lumen of the large bowel and an otherwise typical exudate will show no living organisms. Under such circumstances a high proctoscopic examination may reveal typical crateriform ulcers, from the centers of which living amebae may be obtained. It must be emphasized, however, that only about 37 per cent of amebic lesions occur in the rectum and that the greatest number of primary lesions occur in the cecum and appendix, which cannot be examined with a tube. Cysts of *E. histolytica* are not passed in a liquid exudate, nor does encystation occur in such a medium after it has been expelled from the bowel. Charcot-Leyden crystals are frequently seen in typical amebic dysentery exudate.

The encysted stage is found in formed stools of both carrier cases and those having an intermittently formed and liquid stool. These cysts are typical (Figs 1 C-1 F). They are spherical bodies, 9 to 16 μ in diameter, with a thin cyst membrane, finely granular cytoplasm, and one to four nuclei, depending on their degree of ripeness. These nuclei are characteristic, with a fine chromatin dot (karyosome) in their center suspended in a stellate net of achromatic fibrils, and a peripheral chain of beadlike or plaque-like chromatin particles just within the nuclear membrane. There are no red blood cells or other undigested food particles within the cyst, these have all been expelled just before encystation occurs. However, in about 10 per cent of the cysts one or more sausage-shaped chromatoidal bodies are found in the cytoplasm. These have a chromatin staining reaction and are regarded as excess chromatin extruded from the nucleus at the time of encystation.

In semiformed stools precystic forms of *E. histolytica* are not infrequently found. These (Fig 1 B) are uninucleate rounded-up specimens, from which the undigested food has been or is about to be extruded, but as yet without a cyst wall. If the stool is allowed to desiccate they will frequently encyst.

In examining for *E. histolytica* in amebic hepatitis it should be remembered that the organism is not commonly found in the material aspirated from the "abscess" cavity, but lives in the wall of the pocket and can be aspirated out after the pocket has been drained. Only trophozoites occur in this location.

Some workers prefer to place a portion of each sample suspected of harboring *E. histolytica* in a culture medium and incubated at 37° C for twenty-four to forty-eight hours. Probably the most satisfactory medium is that of Boeck-Drbohlav (1925), consisting of a slanting egg substratum and a supernatant layer of Locke's solution (serum-egg albumin). The amebae grow best along the surface of the solid substratum. However, this technic can never take the place of the routine examination of gross and concentrated samples.

Craig (1927) has introduced the use of a complement fixation test both as a routine and a check for microscopic examination for *E. histolytica*. This is a valid method in so far as there is a systemic reaction in the patient harboring the organism.

E. histolytica may be expected in 5 or more per cent (up to 50 per cent) of the population of any given area in the United States, the amount of the infection depending on both latitude and local sanitary conditions.

CAUDAMOEBIA SINENSIS (Fig 2 A-2 B)

This organism has been found in four cases of acute amebic enteritis in native patients in Peking, China (Faust, 1923). Only the trophozoite stage has been seen. It is distinguished by having an almost constant flowing movement from the forward end of the organism, and at the opposite end a small but distinct caudal process. The nucleus has a stellate karyosome and lacks the distinct peripheral chromatin beading of *E. histolytica*.

ENDAMOEBIA GINGIVALIS (Fig 3)

This organism lives in suppurative pockets in the gums, particularly in persons having pyorrhea. Scrapings of the gums around carious teeth fre-

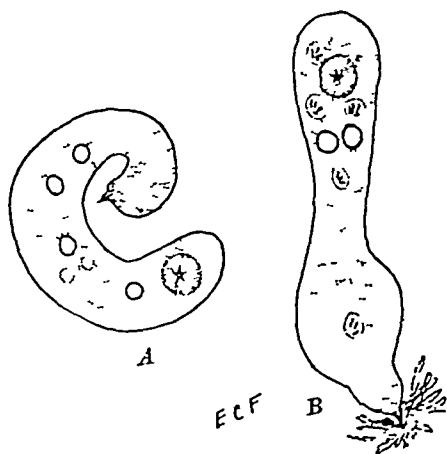


Fig 2

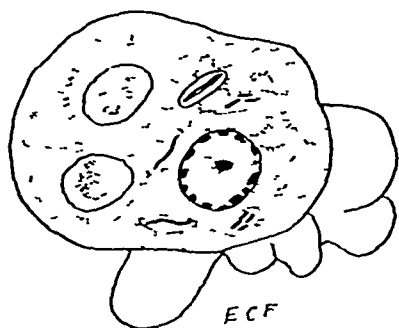


Fig 3

Fig 2—*Caudamoeba sinensis*. Trophozoites showing characteristic structure and movement. $\times 1300$

Fig 3—The active *Endamoeba gingivalis*. $\times 2000$

quently yield this organism, which is fairly active at body temperature. The pseudopodia are limpid clear but small, there is a tendency for several to be extruded at one time. These amebae range in size from 10 to 40 μ and have a narrow marginal ectoplasm and a highly vacuolated granular endoplasm. The nucleus has a karyosome, usually central in position and a ring of chromatin granules just within its membrane. Food vacuoles contain bacteria and white cell casts, possibly also red blood cells. Although the organism probably has an encysted stage it is doubtful if the cyst has ever been observed.

ENDAMOEBIA COLI (Fig 4 A-4 E)

Endamoeba coli is the most common protozoan found in the stool. This organism may be seen in the trophozoite stage in diarrhetic stools or after a saline purgation (Fig 4 A). It varies in size from 15 to 50 μ . The ectoplasm is grayish green and is very granular. The movement is sluggish and little difference can be found between ectoplasm and endoplasm. Usually there are several food vacuoles containing bacteria and vegetable cells. The nucleus is large and is readily seen in unstained specimens. The karyosome is either a single eccentric mass or is composed of a clump of several chromatin granules. There is a peripheral band of coarse chromatin granules. In iodine-stained films the trophozoite frequently contracts into a dense almost opaque mass.

The cyst (Fig 4 B-4 E) is spherical or subspherical, varies in size from 10 to 33.5 μ , has a relatively thick capsule and a dense cytoplasm, in the midst of which can be seen the distinct nuclei (1 to 8 or more, depending on their stage of ripeness). These nuclei are best observed in iodine-stained films. If five or more nuclei can be counted (Fig 4 D-4 E), there is reasonable certainty that the organism is *Endamoeba coli*, although rarely up to 8 nuclei have been found in *E histolytica* cysts. In unripened cysts with 1 to 4 nuclei, it is necessary to determine the nuclear structure. Occasionally chromatoidal bodies occur, these (Fig 4 D) are falciform or splintered structures, very different from the chromatoidals of *E histolytica* cysts. From time to time *E coli* cysts show

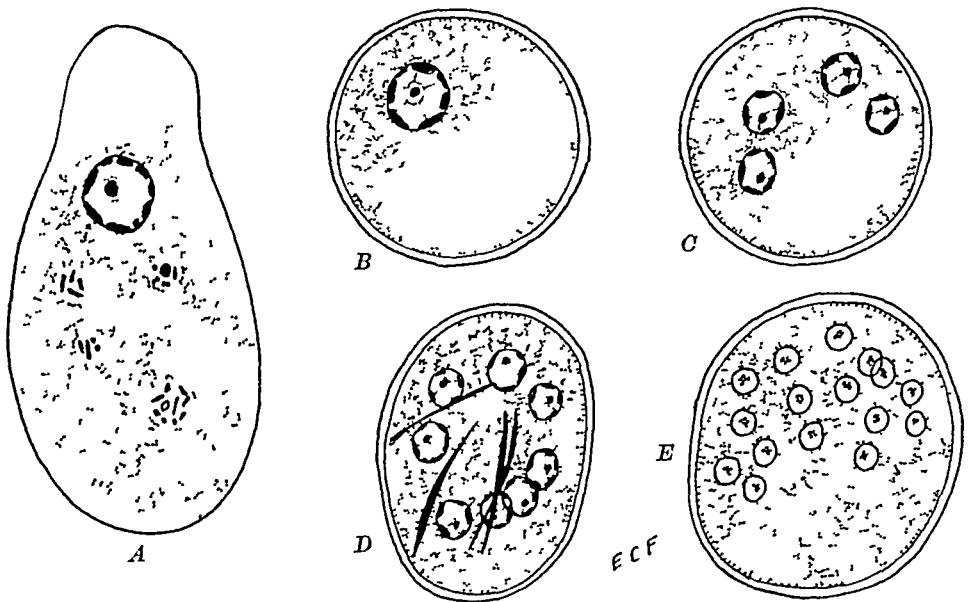


Fig 4—*Endamoeba coli*. A trophozoite B-E representative cysts $\times 2000$

dense diffuse glycogen masses (Fig 4 B-4 C), which obscure the other structures in the iodine stained preparation. The *Councilmanella laffeyi* of Kofoid and Swezy (1923) is regarded by most workers as an aberrant form of *E coli*.

ENDOLIMAX NANA (Fig 5 A-5 D)

This little ameba is a common inhabitant of the intestinal tract. In diarrhea or semiformed stools, it may be seen in the trophozoite stage with granular vacuolated endoplasm and clear limpid ectoplasm, the latter frequently thrown out as hyaline pseudopods (Fig 5 A-5 B). The nucleus of the motile stage is not usually seen in unstained specimens. The cyst (Fig 5 C-5 D) is oval or less commonly rounded (6-10 μ), has a distinct wall, a distinctly vacuolated cytoplasm and 1 to 4 nuclei, which look like punched out holes with a laterally disposed chromatin clump. The chromatoidal masses in the cytoplasm are small and frequently crescentic. Diffuse glycogen masses in the cytoplasm are not uncommonly seen (Fig 5 D).

IODAMOEBIA BUTSCHLI (Fig 6 A-6 D)

This species is never as commonly encountered as *E. histolytica*, *E. coli* and *Endolimax nana*. It is most usually observed in formed stools. The trophozoite (Fig 6 A) varies in size from 5 to 20 μ , is sluggish, the cytoplasm is finely granular and the nucleus which is seen with difficulty in unstained specimens has a large chromatin blob surrounded by a more or less stellate frame of "peripheral chromatin". The cysts (Fig 6 B-6 D) are frequently referred to as "iodine

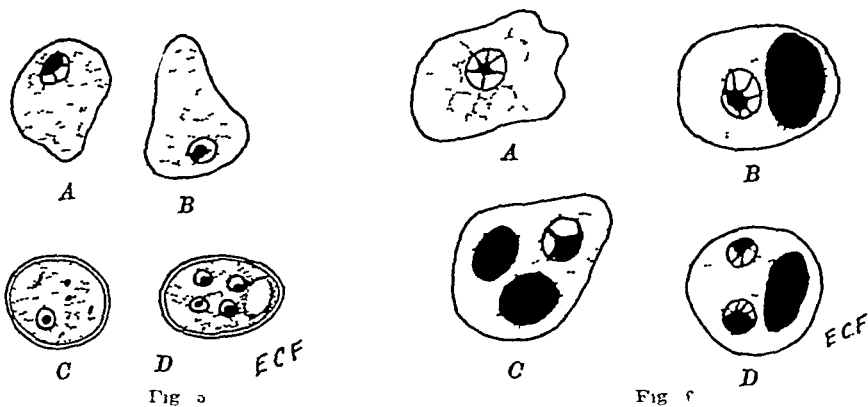


Fig 5—*Endolimax nana* A B trophozoites C D cysts $\times 2000$
Fig 6—*Iodamoeba butschli* A trophozoite B-D cysts $\times 2000$

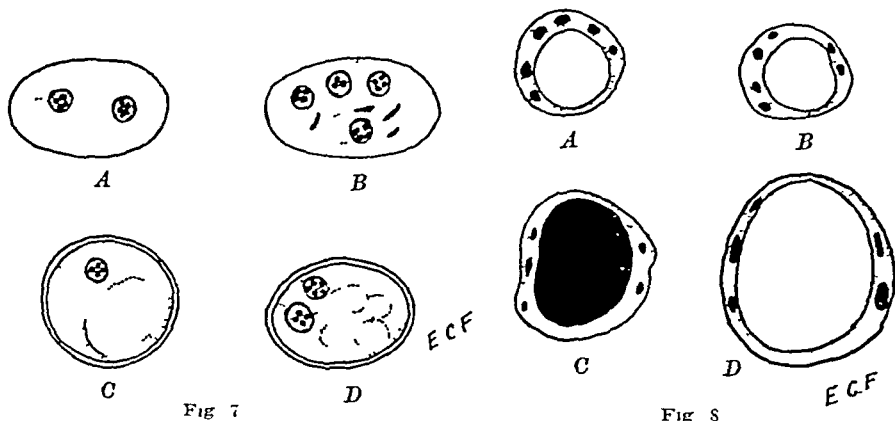


Fig 7—*Dientamoeba fragilis* A B trophozoites C D cysts $\times 2000$
Fig 8—Representative specimens of *Blastocystis hominis* from human feces $\times 2000$

cysts because of the large definitely delimited glycogen mass which stains a mahogany brown in the iodine treated preparation. They are irregularly oval or rounded and vary in size from 6 to 17 μ and have a distinct capsule. There is usually only one nucleus which is similar to that of the trophozoite stage. The cytoplasm has one or more vacuoles but no chromatoids have been described.

DIENTAMOEBIA FRAGILIS (Fig 7 A-7 D)

This rare amoeba is usually found to have two nuclei in the trophozoite stage (Fig 7 A-7 D) although uninucleate and quadrinucleate active forms have been observed. The organism ranges from 3.5 to 12 μ in size, is actively motile

and has clear lobate or sinusate pseudopodia. The endoplasm is granular and contains food vacuoles. The structure of the nucleus is characteristic, with a delicate membrane, and a collection of several distinct karyosomal granules near the center. Uninucleate and binucleate cysts (Fig 7 C-7 D) have been observed by Kofoid, but the organism is usually so delicate that it degenerates rapidly in passed stools.

Councilmania tenuis, *C. dissimilis* and *Karyamoebina falcata*, forms described by Kofoid and his colleagues (1924, 1928) apparently have not been observed by other workers.

BLASTOCYSTIS HOMINIS (Fig 8 A-8 D)

Although this yeastlike organism is in no wise related to the amebae, it is frequently confused with them by the unsuspecting diagnostician. These organisms are spherical, colorless, highly refractile and measure from 5 to 30 μ in diameter, with a considerable size variation in any particular specimen of stool. They consist of a vacuolated center surrounded by a thin peripheral rim of cytoplasm with one or more nuclei. The body is enclosed in a relatively impermeable capsule. At times dividing dumbbell forms are seen. These organisms frequently shrink in the iodine-stained films.

Blastocystis is most commonly found during the season when fresh fruits such as grapes, apples and pears, are eaten raw. This organism is a most serious contaminator of *E. histolytica* cultures.

2 Flagellates (Class Mastigophora)

The flagellate protozoa found in the gastrointestinal tract of man include *Trichomonas hominis*, *Trichomonas buccalis*, *Chilomastix mesnili*, *Giardia lamblia*, *Embadomonas intestinalis*, *Embadomonas sinensis*, *Enteromonas hominis*, *Tricricomonas intestinalis*, *Cercomonas longicauda* and *Bodo caudatus*. Of these species *Trichomonas hominis*, *Chilomastix mesnili* and *Giardia lamblia* are the common intestinal forms, *Trichomonas buccalis* lives in the mouth, and the other species are without doubt coprophagous nonpathogenic organisms seen from time to time in diarrheic stools. In addition, *Trichomonas vaginalis* occurs in a fairly high percentage of specimens of urine from female patients and is occasionally found in catheterized urine of male patients.

TRICHOMONAS HOMINIS (Fig 9)

This flagellate is a comparatively common parasite, growing for the most part in the upper levels of the large bowel. It is most commonly observed in diarrheic or semiformed stools and is apparently associated with low gastric acidity, which permits passage of the trophozoite stage through the stomach without injury to the parasite. The organism does not have a cystic stage. The active organism is more or less heart-shaped, with a length varying from 7 to 20 μ and a breadth of 3 to 7 μ . Its marked motility is due to the combined action of its flagella, undulating membrane and plastic body. Forms have been described with three anterior flagella (*Tritrichomonas*), four anterior flagella (*Tetratrichomonas*) and five anterior flagella (*Pentatrichomonas ardin delteih*). Behind the base of these flagella on the ventral side is a slitlike buccal cavity (cystostome). Arising from the point of insertion of the flagella and descend-

ing along the dorsal side in a slightly spiral course, is the undulating membrane which ends near the posterior end of the body. Attached to its margin is an accessory flagellum, which at times has a free trailing posterior end while along its inner margin is an axoneme. Arising just behind the flagellar origin and continuing through the body distalwards to break through the posterior end is the semirigid spikelike axostyle. An oval nucleus may be seen near the anterior end of the organism. The cytoplasm is finely granular and contains numerous vacuoles.



Fig 9

Fig 10

Fig 11

Fig 9—*Trichomonas hominis* $\times 2000$

Fig 10—*Trichomonas buccalis* $\times 2000$ (Adapted from Kofoid)

Fig 11—*Trichomonas vaginalis* $\times 2000$ (Adapted from Kunstler)

Trichomonas hominis moves with a nervous jerky, somewhat corkscrew forward motion. The undulating membrane, which can be seen best when the animalcule is more or less quiescent, is diagnostic for the genus. Multiplication is by longitudinal binary fission.

TRICHOMONAS BUCCALIS (Fig 10)

This organism ($7-12 \mu$ by $4-8 \mu$) lives and breeds in the tartar around carious teeth and in pus pockets in cases of pyorrhea. It has four anterior flagella and a delicate axostyle which barely protrudes beyond the posterior end of the body. The nucleus is a large oval body. The undulating membrane has a decided twist near the midbody level.

TRICHOMONAS VAGINALIS (Fig 11)

This large flagellate species is found in vaginal smears and catheterized urine of a fairly high percentage of women and is occasionally found in catheter-

ized urine of the male subject. It measures 15 to 25 μ in length by 10 to 15 μ in breadth. There are four flagella arising from the slightly protuberant anterior end. The undulating membrane spirals to the posterior end of the animalcule but has no free trailing flagellar termination. The weakly developed axostyle likewise has no free termination. The distal end of the organism is acutely pointed and often curved.

CHILOMASTIX MESNILI (Fig. 12 A-12 B)

This flagellate organism is found in both diarrhea and solid feces. In the former medium it is usually in the trophozoite stage although when the material

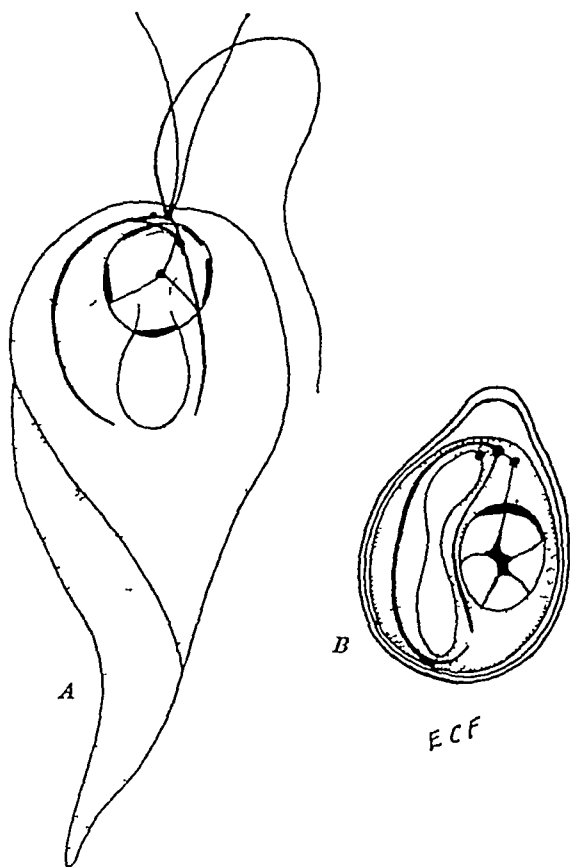


Fig. 12—*Chilomastix mesnili*. Left trophozoite, right cyst. $\times 4000$

is centrifuged encystment frequently results from the mechanical agitation, in the formed specimen cysts are the usual stage observed. The motile form (Fig. 12 A) varies from 6 to 24 μ in length and 2 to 9 μ in transsection. The anterior end is oval and the posterior end is drawn out into a plastic caudal process. There is a pronounced counterclockwise torsion to the body. The conspicuous cytostome or mouth begins at the anterior end on the ventral aspect and extends backward as a dumbbell-shaped cleft of the cytoplasm toward the midplane of the body. Projecting from the anterior end are three delicate flagella. The large oval or subspherical nucleus can rarely be seen without hematoxylin staining.

The cytoplasm is hyaline finely granular and contains several vacuoles. The organism exhibits an active jerky, spiral movement, during which the caudal process may elongate considerably. Reproduction is by longitudinal fission.

The encysted stage (Fig 12 B) is characteristically lemon-shaped. It measures from 6.5 to 9 μ in length by 4.5 to 6 μ in breadth. Iodine stained specimens

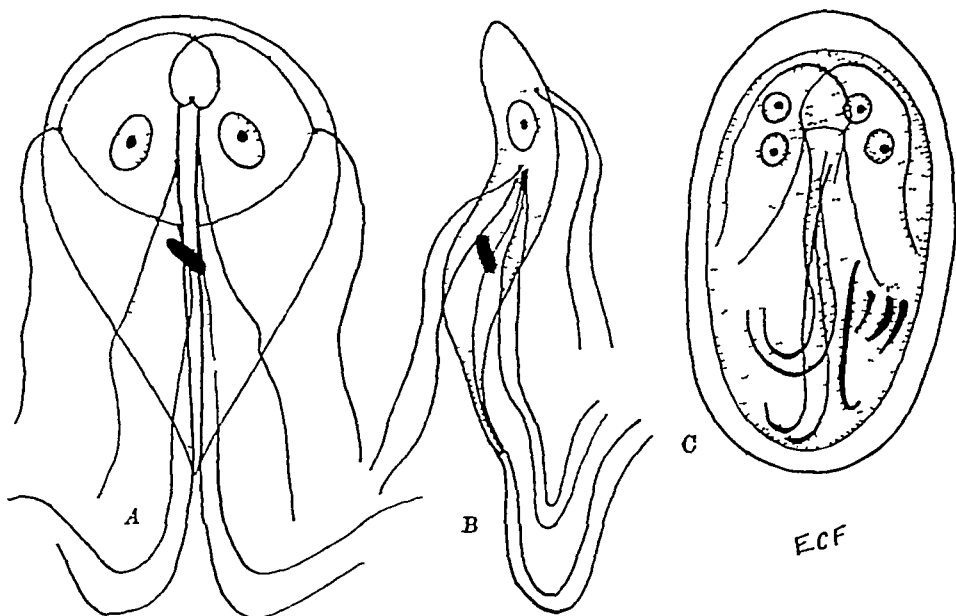


Fig 13—*Giardia lamblia*. Left ventral view of trophozoite center lateral view of trophozoite right cyst. $\times 6000$

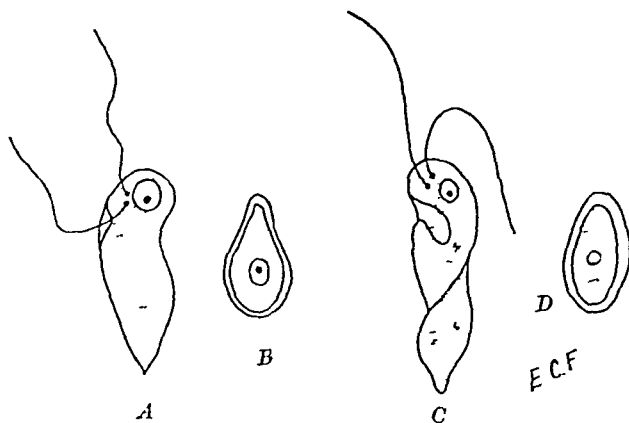


Fig 14—*Embryomonas* spp. A trophozoite and B cyst of *E. intestinalis* C trophozoite and D cyst of *E. sinensis* $\times 2500$

show evidence of the cytostome and retracted flagella as well as a definitely outlined enveloping cystic capsule.

GIARDIA LAMBLIA (Fig 13 A-13 C)

In its trophozoite stage (Fig 13 A and 13 B) which is found only in unformed feces, this flagellate exhibits a very rapid movement produced by the 4 pairs of flagella arising from the ventral side of the body. The organism is

top-shaped in contour, with a rounded anterior and a pointed posterior end. Its dorsal side is rounded and its ventral aspect cupped, so that on lateral view (Fig 13 B) it looks something like a half pear which has been cored. The size ranges from 9 to 20 μ (length) by 5 to 12 μ (breadth). The cytoplasm is finely granular and two nuclei can usually be seen.

The cyst (Fig 13 C), which is the stage of the organism most usually seen, is oval, measuring 8 to 14 μ long by 6 to 10 μ broad. The cytoplasm is definitely

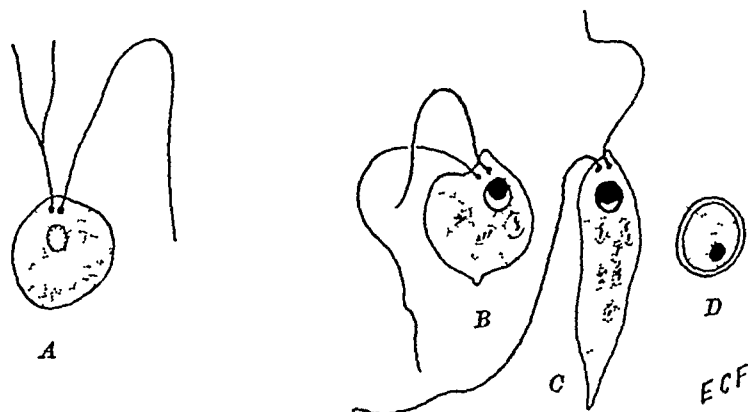


Fig 15—4 *Enteromonas hominis*. B, C trophozoites of *Bodo caudatus*. D cyst of *B. caudatus*. $\times 2000$

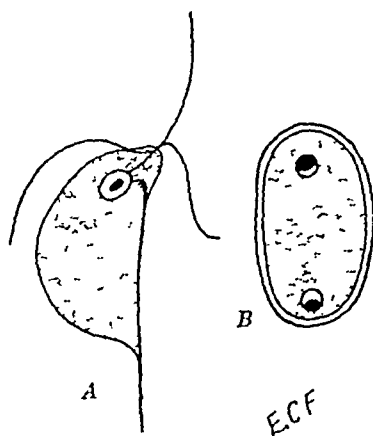


Fig 16—*Tricomonas hominis*. Left trophozoite, right, cyst. $\times 2000$ (After Wenyon and O'Connor)

separated from the enveloping thin-walled hyaline capsule. Usually four spherical nuclei can be observed as well as the retracted flagella. There are also short thick fibrils, which may be associated with the process of reproduction. These cysts have a low specific gravity and are not readily concentrated by centrifuge methods. They stain acceptably with the Donaldson's iodine technique.

EMBADOMONAS (WASKIA) INTESTINALIS AND L. SINENSIS (Fig 14 A-14 D)

These minute organisms ($4.5-6 \mu \times 2.5-4 \mu$) are uncommon compared with *Trichomonas*, *Chilomastix*, and *Giardia*. The trophozoite stage (Fig 14 A, 14 C) is irregularly ovate, biflagellate and has a slitlike cytostome. One flagellum

frequently trails backward across the cytostome. The cyst (Fig 14 B, 14 D) is pyriform ($4.5-6 \mu \times 3 \mu$) and easily confused with yeast cells. These coprophagous species are most commonly found in diarrheic stools of persons having a gastrointestinal upset of a chronic or acute nature. I have seen *E. intestinalis* once in catheterized urine from a female patient.

ENTEROMONAS HOMINIS AND BODO CAUDATUS (Fig 15 A-15 D)

These minute subspherical or oval biflagellate species occur in diarrheic stools of persons having a digestive upset. Their minute cysts are easily confused with yeasts. These are not uncommonly seen in contaminated stools.

TRICERCOMONAS HOMINIS (Fig 16 A-16 B)

The trophozoite of this small flagellate ($4-8 \mu$ in transverse diameter) has 3 anteriorly directed and one posteriorly trailing flagella. The organism is ir-

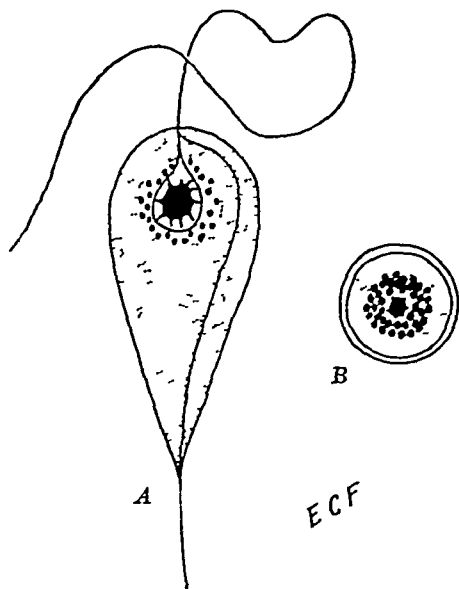


Fig 17—*Cercomonas longicauda*. Left trophozoite, right cyst. $\times 2000$ (After Wenyon)

regularly oval, at times with a suggestion of a caudal projection. The cyst (4.8μ) is oval and usually binucleate. This organism occurs occasionally in diarrheic stools.

CERCOMONAS LONGICAUDA (Fig 17 A-17 B)

The trophozoite of this small flagellate ($5-16 \mu$) is pyriform, has a single very long anteriorly directed flagellum and a posterior one which extends considerably beyond the posterior end of the body. There are numerous granules surrounding the anteriorly disposed nucleus. The cyst ($4-7 \mu$) is subspherical and shows the granules around the nucleus. This organism is found occasionally in diarrheic stools.

3 The coccidia (Class Sporozoa)

Only one species of this group has been definitely proved to be a human intestinal parasite. This is *Isospora hominis*.

ISOSPORA HOMINIS (Fig 18 A-18 B)

The organism lives in the villi of the small intestine of man, where schizogony and the sexual products develop and fertilization takes place. The presence of the parasite produces a diarrhea, which persists until the parasites are spontaneously evacuated. The stage recovered from the stool is the oocyst. These are heavily walled hyaline bodies, measuring 25 to 33 μ in length by 12 to 16 μ in cross section. They are irregularly elongate oval in shape and have a delicate capsular pore at one end. The capsule is very resistant to stains. The immature cyst (Fig 18 A) has a single blastomere. In older stools this divides into two, and four internal spores of a curved sausage shape are formed from each of these (Fig 18 B). In the five cases which I have personally seen, the cysts disappeared from the stools in two or three weeks after the onset of the diarrhea, in other words, as soon as the infection spontaneously cleared up.

4 Ciliates (Class Ciliata)

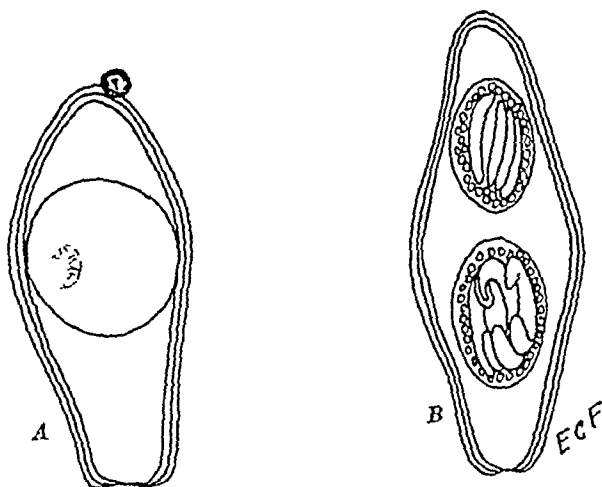


Fig 18—*Isospora hominis*. Left cyst from freshly passed feces right mature cyst from cultured feces $\times 2000$

Only one form of importance is found parasitic in man. This is *Balantidium coli* from the large bowel.

BALANTIDIUM COLI (Fig 19 A-19 B)

This large infusorian form is associated with a pathologic process paralleling that of *E. histolytica* in the cecum, appendix, colon, and rectum. It is found in man most commonly in the tropics where monkeys are uniformly infected. In temperate zones the pig is an important reservoir of the infection. The trophozoite (Fig 19 A) is oval, slightly attenuated at the anterior end and measures from 30 to 120 μ in length by 20 to 50 μ in greater transsection. An anterolateral invagination leads into a distinct cytostome or mouth cavity. The entire body is covered with many cilia, arranged in spiralled rows. These cilia are the locomotor organs. Internally there is a large kidney shaped trophonucleus and a small kinetonucleus which lies in the hilum of the trophonucleus. There are also food vacuoles containing ingested starch and other food particles. The cyst (Fig 19 B) is spherical, has a well-defined capsule and has a diameter about

that of the greater breadth of the trophozoite. Trophozoites are most common in unformed specimens. Care should be taken not to confuse this organism with numerous infusoria which get into fecal specimens from contamination.

II. PROTOZOA OF THE HEMATOPOIETIC ORGANS, BLOOD STREAM AND OTHER BODY TISSUES

1. *The hemoflagellates (Class Mastigophora, Family Trypanosomidae)*

This group includes the three species of *Leishmania*, *L. donovani*, *L. tropica*, and *L. americana* and the three human trypanosomes, *Trypanosoma gambiense*, *T. rhodesiense* and *T. cruzi*. These forms are usually diagnosed from thin blood or pulp smears, dried and stained with Wright's or Giemsa's stain.

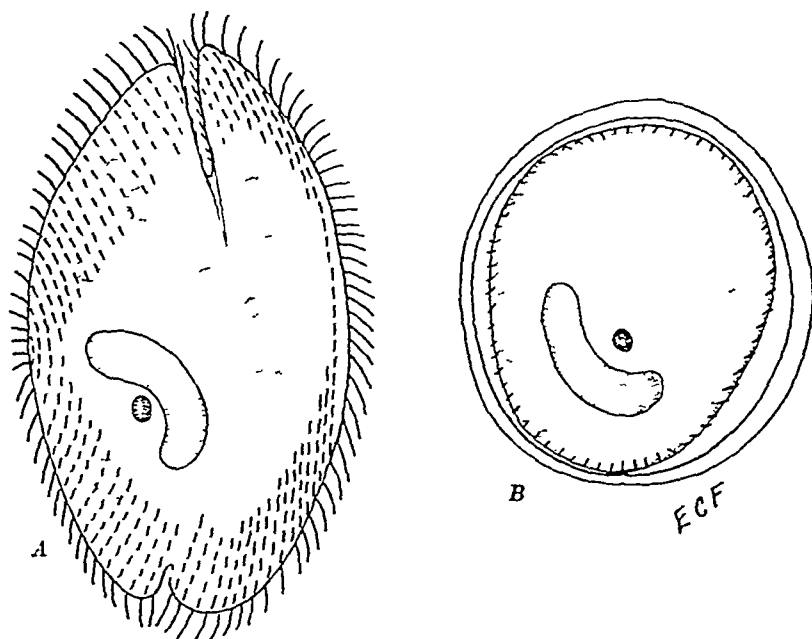


Fig 19—*Balantidium coli*. Left trophozoite, right cyst. $\times 1000$

LEISHMANIA DONOVANI (Fig 20 A-20 F)

This species is the causative organism of kala azar, a visceral leishmaniasis producing a hyperplasia of the lymphoid tissue of the hematopoietic organ and the reticuloendothelial system. In the human body the organism is a small oval or torpedo-shaped body parasitic in the cytoplasm of the endothelial monocytes and wandering cells (Fig 20 A) and is occasionally found free in the circulating blood. It measures 2 to 5 μ in length by 1.5 to 2.5 μ in transverse diameter. The cytoplasm stains a delicate blue tint, the trophonucleus a delicate lavender or violet, and the rod-shaped blepharoplast, which lies at right angles to the trophonucleus, takes a dark reddish violet or madder hue. The most ready diagnosis is made from splenic or hepatic pulp smears, although biopsy of subcutaneous tissue may locate the parasites. In smearing tissue pulp the large heavily parasitized monocytes frequently rupture, and the minute parasite will be found spread out in a fan-shaped area on one side (Fig 20 B). Frequently only the

triphonucleus and the blepharoplast are well stained. Blood films alone are much less likely to have the organisms. Since kala azar is seldom an acute infection some diagnosticians prefer not to risk spleen or liver puncture and depend on culture of the organism from the monocyte layer of centrifuged blood cells, the culture being made on N N N media, which is incubated at about 20

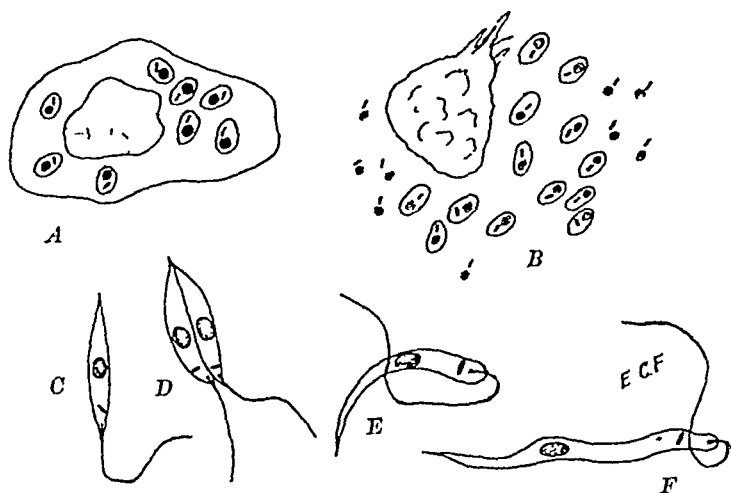


Fig 20—*Leishmania donovani*. A, B leishmaniform stage from endothelial cells of spleen. C-F, flagellate stage from culture. $\times 2000$

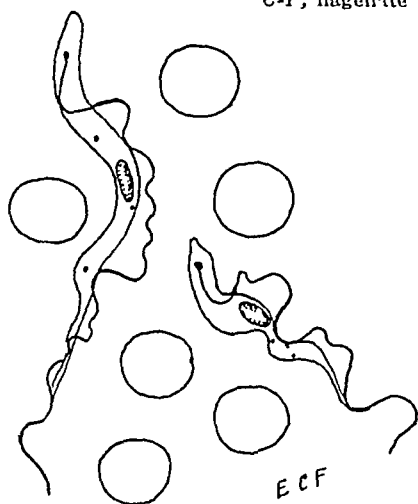


Fig 21

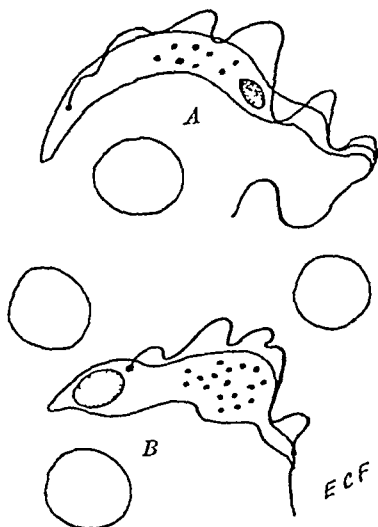


Fig 22

Fig 21—*Trypanosoma gambiense* in peripheral blood film. $\times 2000$

Fig 22—*Trypanosoma rhodesiense* in peripheral blood film. A usual form. B posterior nucleate type from rodent host. $\times 2000$

22° C for three days or more. The flagellate stage of the organism (Figs 20 C-20 F) is found in the water of condensation of the culture (Young and Van Sant, 1923)

Due to an excess of serum euglobulin in the blood plasma of kala azar patients the addition of distilled water to a blood sample (20 c mm of blood,

0.6 c.c. aq. dist.) results in a flocculent precipitate in positive cases (Sia, 1921). There is also a positive aldehyde test when 1 c.c. of blood serum of a patient is treated with a drop of strong commercial formaldehyde (Napier, 1922). These tests are specific except in areas where *Schistosoma* infections are also prevalent. A marked leucopenia with a relative lymphocytosis is a common blood picture in kala azar.

LEISHMANIA TROPICA

This organism, which is structurally similar to *L. donovani*, produces Oriental sore (Aleppo button, Delhi boil), a cutaneous infection in which the organisms are found in endothelial cells, large monocytes and occasionally in polymorphonuclear leucocytes. Smears of the infected tissue properly stained, show these parasites in much the same way as splenic pulp smears reveal *L. donovani* forms. The organism may be cultured on NNN media.

LEISHMANIA AMERICANA

This organism, which is structurally similar to *L. donovani*, produces a mucocutaneous infection. The organism can be recovered from the lesions in a similar manner to those of the two preceding species.

TRYPANOSOMA GAMBIENSE (Fig. 21)

This hemoflagellate form is the causative organism of West African or Gambian sleeping sickness, which is characterized primarily by a hyperplasia of lymph gland tissue. It can be recovered from the peripheral blood, enlarged lymph gland pulp or in later cases from sedimented spinal fluid of an infected patient. The parasite in this stage is typically trypaniform, is roughly spindle-shaped, with a narrowly pointed nonflagellar end and an attenuate flagellate end. Spiraling along one side is the undulating membrane, on the margin of which the main portion of the flagellum is attached. The organism measures 16 to 30 μ in length by 1.5 to 2.5 μ in breadth. Near the middle of the body is the large oval trypomastix nucleus, while the flagellum arises from a basal granule very near the blepharoplast, at the nonflagellar end of the body. The organism divides by longitudinal fission. An intermediate stage occurs in tsetse flies, which serve as transmitting agents. White rats and mice serve as good laboratory animals for the infection. In infected animals a trypanolytic substance is developed which is specific for *T. gambiense*. This criterion serves to differentiate this species from *T. rhodesiense*. The organism can be cultured on NNN media. In infected individuals there is a marked anemia and a relative lymphocytosis.

TRYPANOSOMA RHODESIENSE (Fig. 22)

This organism is the causative organism of Rhodesian sleeping sickness. In man it is practically impossible to distinguish this species from *T. gambiense*. In laboratory animals (rat, guinea pig, rabbit) and rarely in man, there are, in addition to the usual forms, posterior nuclear forms (Fig. 22 B) which serve to differentiate the species. This infection is much more rapidly fatal in laboratory animals as well as in man than is *T. gambiense* infection. Specific trypanolytic and agglutinin substances are also elaborated in this disease.

TRYPANOSOMA CRUZI (Fig 23)

This organism is the causative organism of Chagas disease or South American trypanosomiasis. The organism in peripheral blood (Fig 23 A) has the typical trypaniform characteristics, it is, however, dimorphic, one form being long and slender, with an elongate trypomastix nucleus and one being shorter, with an oval or nearly spherical nucleus. The flagellum both in its attached and free portions, is much coarser than that of the African species of man. The parasite does not undergo longitudinal division in the peripheral blood of man. In the tissue, including the heart muscle, lymph glands and brain, *T. cruzi* assumes a leishmaniform (Fig 23 B) as well as a trypaniform type. There is no marked anemia in the disease although a leucocytosis is characteristic. Laboratory animals can be infected with some difficulty. Transmission is produced by bites of *Triatoma megista* and related species of bugs.

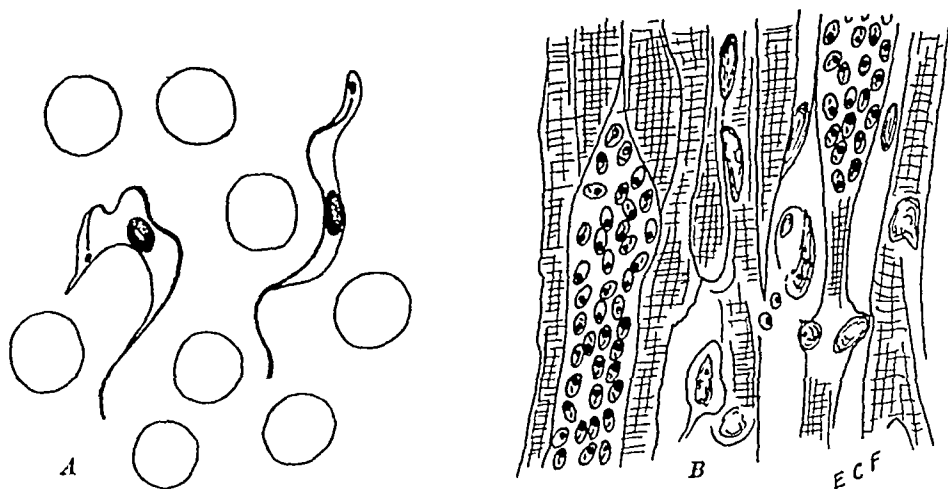


Fig 23—*Trypanosoma cruzi*. A, flagellate stage in peripheral blood. B, leishmaniform stage in heart muscle. $\times 2000$. (Adapted from Chagas.)

2 The malarial parasites (Class Sporozoa, Order Hemosporidia, Family Plasmodiidae)

Three species of this group are parasitic in man, *Plasmodium vivax* (the organism of tertian malaria), *P. malariae* (the organism of quartan malaria) and *P. falciparum* (the organism of estivo-autumnal or malignant malaria). The asexual phase develops in man and the sexual phase in the appropriate female anopheline mosquito. It is important not only to differentiate the three species from one another but also to distinguish the several stages of development of each species in the blood.

DIFFERENTIAL CHARACTERISTICS OF THE MALARIAL PARASITES

The usual method of examining blood films for the malaria parasites, as for other infections of the blood stream, is by the *thin blood film*. Practically all of the preparations of so-called typical malaria parasites are those seen in the

thin film. It seems appropriate therefore, to take up the various stages of the three species of malaria parasites from this point of view.

In each one of the three species, the earliest stage which will be found in examination of the thin blood film is the young growing stage or early trophozoite. In all three species, it is a small rounded body staining azure blue with Wright's or other Romanowsky stains, and having on one side a small brick-red chromatin or nuclear dot.

The Tertian Parasite (Plasmodium vivax)—In the tertian trophozoite (*Plasmodium vivax*) growth ordinarily takes place from the side opposite the nuclear dot either as a dense cytoplasmic mass or with the development of plasmodial processes extending into the red blood cell. This growth continues until the parasite has almost completely filled the red blood cell. Meanwhile the cell has become enlarged and pale, and a considerable amount of hematin pigment has accumulated inside of the parasite. Likewise, in the red blood corpuscle outside of the parasite small orange colored dots, known as Schuffner's dots, are usually elaborated. When this trophozoite has increased to its full extent, so that it has almost completely filled the enlarged pale red blood cell the nucleus divides (process of schizogony) into two, then into four, and subsequently into a larger number of units, so that eventually there will be as many as sixteen to twenty-four of these nuclei that are more or less symmetrically arranged around a residual center containing hematin pigment. This ripe schizont is frequently spoken of as the rosette stage. The red blood cell soon bursts and individual units (merozoites) erupt into the blood stream, each one in turn infecting another red blood corpuscle. This asexual multiplicative process takes place time and again during the incubation or prepatent period, and also to a certain extent after symptoms develop.

As soon as the system of the parasitized individual begins to elaborate antibodies, the malarial parasite, in order to protect itself, also develops gametocytes from some of the trophozoites. These have a more regular outline, being more or less oval. The nuclear dot is somewhat less distinct than it is in the ordinary trophozoite stage, and a larger amount of the hematin pigment is scattered throughout the parasite. One can recognize both male and female types of this mother sexual cell, the difference being that in the male the nuclear material is more scattered, and therefore stains less definitely. These female and male gametocytes are the latest stages found in the human body. Subsequent stages develop only after the gametocytes get into the stomach of the acceptable female anopheline mosquito.

The most important diagnostic feature to recognize in the tertian parasite is the enlarged parasitized cell, which becomes very definitely pale as its substance is taken up by the parasite.

The Quartan Parasite (Plasmodium malariae)—The parasitized red blood cell does not increase in size. Occasionally it is even somewhat shrunken. It does not become paler in color. Frequently it is a bluish slaty hue, but this condition does not develop until the parasite has increased somewhat in size. The quartan parasite never gets as large as the cell which it parasitizes. There is more of a tendency also for the vacuole to be small compared with the amount

of solid cytoplasm. When the process of schizogony begins, the number of units which develop is few, seldom more than eight, compared with the larger number in both of the other two species. These nuclei usually have a much more symmetrical arrangement than they do in tertian infection, so that they appear like the petals of a rose around the center. Meanwhile, as in the other two species, a considerable amount of hematin pigment is deposited within the quartan plasmodium.

The so-called banded form, another variety of the quartan trophozoite is relatively common, although not as common as it is figured in the textbooks. It may develop directly from the merozoite which has just entered the red blood cell, it continues to grow by a widening of the band so as finally to occupy a total of from two-fifths to one-half of the area of the red blood cell. The gametocyte stages of the quartan plasmodium are ovoid bodies, more nearly filling the parasitized red blood cell than the trophozoite stages. They have a large amount of pigment scattered throughout their cytoplasm. The female gametocyte has a definite nucleus, while the male gametocyte may have nuclear material so scarce and so scattered that it is difficult to recognize it in the ordinary stained film.

The Estivo autumnal Parasite (Plasmodium falciparum)—There may be from one to several of these trophozoites inside each parasitized red blood corpuscle. Sometimes the ring may be peripheral in position, looking like a minute blister on the side of the cell, with the chromatin dot on the elevation of the blister. This early stage of estivo-autumnal infection is the only asexual form found in ordinary peripheral blood. The later trophozoite stages and the schizont are found almost without exception in the blood of the deeper viscera, including the maternal side of the placenta. Occasionally, however, these later stages in schizogony are found in peripheral blood in which event there is a poor prognosis for the case. The late trophozoite is never as large as even the moderately developed trophozoite of tertian or quartan infection. It is usually oval in shape, quite regular in outline, and has a mass of cytoplasm opposite the nuclear or chromatin element. When it divides, one can recognize from eight to twelve up to a maximum of thirty-two nuclear elements, these being arranged around the center of hematin pigment. In the estivo autumnal parasite, there are found from time to time stipplings in the nonparasitized part of the red blood cell. These are similar in type to those found in the tertian infection, but are called Maurer's dots.

The early gametocyte stages are also not found in peripheral blood, but in the deeper visceral circulation. These early gametocytes of estivo autumnal malaria are broadly oval in shape, and at this stage male and female forms cannot be differentiated, although when they get out into the peripheral blood the two sex types can be distinguished. Both of them when ripe are of the so-called crescent shape with a thin membrane around the parasite which really is the pellicle of the parasitized red blood cell. Within the crescent one can recognize hematin pigment and concentrated nuclear material in the female gametocyte and more diffuse nuclear material in the male gametocyte. At a somewhat later stage, the pellicle of the parasitized red blood cell breaks down and the crescent or bean shaped gametocyte is set free into the blood stream.

THICK BLOOD FILMS

The thick blood film is a method of diagnosing malaria which has been accepted by many laboratorians as much more valuable than the thin blood film. This film is prepared by taking two or three drops of blood from the ear or finger, or in the case of the small child, from the great toe, placing it in the center of a clean slide, and distributing it with the corner of another clean slide, so that it will occupy the area of a quarter, and also so that the center will not be thicker than the margin. This is then allowed to dry thoroughly. In moist tropical climates it is frequently necessary to place it in a drying oven for an hour or so. It is then dehemoglobinized and stained at the same time by using Giemsa's stain. It is however quite possible to use dilute Wright's stain in exactly the same way, by taking the stock Wright's solution diluting it one part to thirty with neutral distilled water. The film is placed in a staining jar and left there for a half hour to an hour. When the film is taken out it is thoroughly washed in water and dried. An examination will indicate that the red blood cells have been dehemoglobinized so that they appear as delicate ghosts on the film, although all of the leucocytes, as well as any malarial parasites which were present in the red blood cells, will be properly stained. The advantage of this method is that one can see right down through several superimposed layers of red blood cells. In other words, you have a concentrate of malarial parasites, which, if they had been stained by the ordinary process, would be so dense that the parasites could not be discovered. The parasites in this type of preparation are not flattened as on the thin film so that it is necessary to acquire some experience with the method before accurate diagnosis can be made.

The thick film is particularly valuable in cases that have been found constantly negative by the thin film method, the writer has been able to vouch for this fact in several cases where the clinical symptoms were those of a malarial infection, but where the thin blood film was consistently negative.

3 *Sarcosporidia* (Class undetermined, possibly *Sporozoa*)

A few cases of infection with a member of this group, *Sarcocystis hominis*, have been found in human muscle tissue. It seems likely that the infection in man is really a casual inoculation from domestic or wild animals which more commonly harbor these species of parasites. The organisms have been recovered from heart muscle, muscle fibers of the larynx, biceps muscle and tongue. They are bean or sickle shaped and occur in large tubular clusters between the muscle bundles. The individual spores may measure up to $16\ \mu$ by $9\ \mu$ and the bundles as much as 25 mm in length. The human cases reported have mostly been determined from necropsy material, although Darling (1919) diagnosed one case from a biopsy specimen.

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INTESTINAL PROTOZOA IN CLINICAL MEDICINE*

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THE position held by intestinal protozoa in clinical medicine is a confused one, commonly varying from one extreme to another among practitioners. The question of the relation of these protozoa to health and disease is of great and world-wide importance.

It is indicated by reports from various and widely separated sources that the general incidence of intestinal protozoa may be conservatively estimated at from 20 to 25 per cent or higher. It appears that the conditions for their transmission are more favorable in the warm countries but reliable studies made in north temperate regions and in institutions with modern sanitary facilities have shown that hot climate and unsanitary living habits are not essential to their spread.

In some sections of this country little or no attention is paid to intestinal protozoa in the diagnosis or treatment of disease. In other places the mere presence of one or more of these organisms is sufficient grounds for a specific diagnosis and therapeutic attack. With some medical men diarrhea or dysentery is the only condition associated with intestinal protozoan parasitism, while others have included among their effects such diseases as iritis, arthritis, Hodgkin's disease, cholecystitis.

It appears that much of the confusion concerning their status results from too much generalization in our consideration of them, to think or speak of them as a class, as protozoa, or as amebas or flagellates or ciliates, is of the same order as would be a similar consideration of bacteria as a class. The necessity of properly identifying and of studying the effects of the individual species concerned should be clearly forced upon us by the definite knowledge that one species of ameba is a disease producer while another is not.

For improvement in the confused status of the intestinal protozoa of man it is necessary to improve the knowledge and familiarity of the medical profession in the subject. Particularly is it important to have improvement in laboratory identification of these organisms. It is a widespread condition that laboratorians, medical and nonmedical, who are not well qualified for this work are given the responsibility of it.

Flagellates may be satisfactorily identified in a fresh liquid stool. The amebas are best identified in the encysted stage, which occurs mainly in the naturally formed stool. Many errors are made in naming an ameba found in liquid stools, of dysentery, diarrhea or produced by a purgative, where it is present usually in the active form. For routine stool examination, unless there is diarrhea, it is best to study first a fresh stool produced by a saline cathartic. If an ameba is found and the cysts are not seen, one who is not an expert protozoologist should wait for its identification in a formed stool when the cyst

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will usually appear. However, in the case of clinical amebic dysentery, with the typical blood tinged mucous stool containing the active amebas with ingested red blood corpuscles, it is not advisable to wait for more accurate identification of the ameba before instituting proper treatment.

THE AMEBAS

Among the amebas occurs the protozoan of outstanding importance in clinical medicine, *Endamoeba histolytica*.

Once considered of importance only in the tropics, this parasite is of world wide distribution. In temperate or colder regions its incidence is usually less but it has been found wherever search has been made for it. The reported incidence in different surveys varies widely from around 1 per cent to 20 per cent and more, the variation in reliable studies apparently depending largely on the nature of the examined people, as to the sanitation of their environment and whether they are well or the subjects of intestinal disease. The greater incidence is naturally where the protection of man from excreta of his own kind is poorest, this usually being in tropical or subtropical regions. In institutional life, with its close personal contact, it is likely to be comparatively high. In rural districts without modern sanitary facilities it is also apt to be high. In modern communities with proper sewage disposal and protection of food and drink it is low. At a rough estimate its general incidence in a country like the United States of America is around 10 per cent. In the modern city it is around 3 per cent, according to my experience. Close personal contact with infected persons and the food handler carrier probably furnish the main means of spread.

The cyst of the organism is the infective stage and prevention of spread of the infection involves prevention of this body from reaching the mouth of man.

When this ameba reaches the intestine of man in viable state, it may produce amebic dysentery or chronic intestinal amebiasis, and there is some indication that it may result in a symptomless carrier state.

The known effects of the organism depend on entry into the intestinal wall with variable penetration, ulceration, and the possibility of transportation through the circulation to near or distant tissues, particularly the liver. A humoral absorption of toxic products of the organism is indicated.

The involvement of the intestine is practically limited to the colon, extension to the lower end of the ileum sometimes occurring. When the penetration and resulting ulceration of the colon are active and extensive, they result in the clinical state of amebic dysentery. This affords the common conception of the results of amebic infection. Amebic dysentery is rather the comparatively uncommon acute phase or end-result of intestinal amebiasis.

What determines the occurrence of amebic dysentery in one infected person and of a symptom producing chronic colitis or of a symptomless state of parasitism in others, or why one of the latter may have an acute flare-up of the infection into dysentery, is unknown. The state of parasitism is constituted of a balance between invasive properties of parasite and resistance of host. When that balance is in favor of the parasite, we have active ulceration and dysentery. When in favor of the host, the disease is halted or held in check or perhaps invasion of the parasite prevented.

The diagnosis of amebic dysentery is not difficult. Its characteristics are frequent bloody mucous stools with abdominal griping and tenesmus. In the stool, on proper examination, are found active amebas, usually containing red blood corpuscles associated with mucus, blood, fecal debris and pus in the advanced stages. These are sufficient for a diagnosis upon which treatment may be instituted.

The diagnosis of chronic intestinal amebiasis is not easy. The patient usually has a history of long-continued ill health with confusing symptoms of many kinds but particularly relating to the intestinal tract "indigestion" as the term goes. Although attacks of diarrhea or even dysentery, alternating with periods of constipation may be discovered in the history, they may not be more conspicuous than in other people of similar ailments. Vague abdominal pains, heaviness, minor tenderness over the colon especially the cecum, flatulence, gaseous eructation, are common complaints. Generally there is underweight, a "tired feeling," a lack of energy, with possibly headaches and joint aches.

Even a working diagnosis can only be made by positive identification of *Endamoeba histolytica* in the stool, possibly only by repeated examination. In practice this amounts to finding and identifying the cyst.*

Since similar chronic complaints are very common and since a large percentage of the people concerned harbor amebas of some species, identification of *Endamoeba histolytica* is the essential for a diagnosis of chronic intestinal amebiasis.

During the course of amebic dysentery or chronic intestinal amebiasis amebic abscess may occur elsewhere, most commonly in the liver.

For a general summary of the treatment of all phases of intestinal amebiasis it may be stated that the measures used should be both specific and nonspecific, that the pathology of the disease should be borne in mind, and that "cure" is not obtained until the ameba is proved to be eliminated, by an extended series of stool examinations.

It is not possible here to go into the details of specific and nonspecific treatment. Emetine in some form, commonly with bismuth, is the "specific" of widest satisfaction, particularly in the acute phases. It is now supplemented by iatren and stovarsol, the former apparently particularly applicable in the acute phase and the latter in the chronic. In the acute phases and during active emetine treatment the patient should be confined to bed and the diet restricted. During treatment of the chronic phase it is not necessary to put the patient to bed, but the diet should be restricted to a bland well-balanced one. A reduction of carbohydrates, sufficient protein, and particular attention to green vegetables and fresh fruits are recommended. There is perhaps more influence of dietary factors in intestinal amebiasis, invasion, resistance and treatment than we now

*Study of amebic cysts for diagnostic purposes in medical practice amounts to looking for that of *Endamoeba histolytica* to the exclusion of the others. Amoeba cysts are rounded, glassy or hyaline bodies easily overlooked, sometimes very numerous, sometimes not. Search for them is facilitated by mixing the particle of stool on a slide with an iodine solution such as Gram's iodine (iodine 1 part, potassium iodide 2 parts, water 100 parts). This stains them various shades of brown and makes them more conspicuous. It also allows the nuclei to become more visible as colorless rings against a yellow-brown background. When a suspicious body is located with the low power lens the higher dry magnification will usually reveal its structure. If the cysts show four round nuclei with tiny central karyosome it is *Endamoeba histolytica*. The main confusion is with the cyst of *Endamoeba coli*; it has eight nuclei. For even better and more prolonged study, fresh smear preparations fixed in warm Schaudinn's solution while still moist and stained by one of the haematoxylin methods is to be recommended. Such preparations exhibit the nuclei much better.

realize In anticipating recovery from this disease we must keep in mind that the organism is to be eradicated, ulcers must be allowed to heal, and constitutional effects be overcome

OTHER INTESTINAL AMEBAS

The main concern about the other intestinal amebas is that they must be differentiated from *Endamoeba histolytica*. They are *Endamoeba coli*, the most common ameba of the intestine, and *Endolimax nana*, *Iodamoeba butschlii*, and *Dientamoeba fragilis*, these three being relatively uncommon and not so subject to the frequent confusion with *Endamoeba histolytica* as is the first. None of these is now under serious suspicion of producing disease.

FLAGELLATES

There are three well-established species of flagellates commonly found in the intestine of man, *Trichomonas hominis*, *Chilomastix mesnili*, and *Giardia intestinalis*. There are others less commonly seen, *Embadomonas intestinalis*, *Tricricomonas intestinalis*, and possibly *Enteromonas hominis*.

Probably the majority of protozoologists most familiar with these organisms now believe the class to be harmless to their hosts, although some hold the question open. Among medical persons there exists the greatest confusion as to the different genera and species and as to their effects on their hosts, a great many practitioners believing that at least some of them are disease producers. To these such terms as "flagellosis," flagellate infection, flagellate diarrhea, and flagellate dysentery relate to clinical states of more or less definiteness in their minds.

There exists no reason why any interested examiner may not easily identify the common flagellates as they occur in the stool and this should be the first objective of those who would study this class of parasitism.

The problem of the effects of these flagellates upon man has reached a stage of *impasse*, when evidence given on either side becomes merely an argument. None of the class has been shown to be an actual tissue invader or destroyer, none has been shown to produce any substance or bring about any state deleterious to the host. There is no positive direct evidence on the question, it is all clinical circumstantial evidence based on the occurrence of indefinite symptoms of abnormal states associated with the presence of the parasites. Such evidence, if it were definite or characteristic, would weigh more than it does. There is no typical or characteristic clinical or pathologic complex to be related to the presence of any of them.

Trichomonas hominis is an inhabitant of the large intestine. It is a common parasite found in fresh stool examination. It occurs from childhood to old age, but is apparently not a parasite of infancy. Continued study of the parasite should be carried on by those in position to do so. Practicing physicians should be careful that they are not led away from making another diagnosis by the finding of this organism in the stool.

Chilomastix mesnili is commonly confused with *Trichomonas*. It is also a common inhabitant of the large intestine. It becomes more common as the age of the individual advances and may live for years in the bowel. It is commonly

found during a state of lowered gastric acidity, or constipation, or the syndrome of chronic cholecystitis, as clinically diagnosed. To consider the clinical aspects of the disease, states commonly associated with its presence may be more to the point than too much attention to the parasite.

Giardia intestinalis is a common inhabitant of the upper small intestine. It is more common in childhood than in later life and decreases in frequency as age increases. In children it is commonly associated with a state of diarrhea, and the younger the child apparently the more commonly this occurs. It is consequently considered to be the cause of diarrhea in children, although studies which indicate this have not been sufficiently well controlled to make this certain. As the age of the host increases the individual seems able to control the parasite and apparently eliminates it commonly.

Its connection with the clinical state of enteritis and diarrhea apparently is lost in older people and the parasite then commonly appears to be harmless. Whether this is by immunization of the host or whether related to the difference in food and in digestive action between early and later life furnish interesting speculation about the unknown.

The diagnosis of this infection depends upon finding the parasite in the stool, although it is occasionally seen in duodenal drainage. In formed stools only the cysts appear. These are small and may be overlooked by the inexperienced examiner. The active form is usually seen in the stool of diarrhea.

THE CILIATES

Balantidium coli is practically the only ciliate of the human intestine, and it is a rare parasite in this country. Its natural host is probably the pig, and human infection probably comes from transmission from this animal. Few authentic cases of balantidial infection have been reported in this country. It has been reported as relatively common in the Philippine Islands and in Central America.

It is easily identified, but is very apt to be confused by the inexperienced with free-living ciliates which may occur in human stools after their passage.

This parasite is a potential and actual disease producer in man, balantidiosis of the intestine of man having a significance akin to intestinal amebiasis.

The acute phase of the disease is the unusual consequence of infection, the acute attack, or the end-result. In this condition the organism is a tissue invader and may give rise to deep ulceration of the colon associated with frequent bloody mucous stools and abdominal griping, but sometimes there is merely a watery diarrhea. Again, there may be ulceration without dysentery or diarrhea.

Much more common than the state of balantidial dysentery is that in which there is a chronic infection without dysentery or even without any apparent disturbance due to it. It is unknown whether in the "carrier" state without symptoms of disease the organism is purely a lumen dweller. The state of infection in which there is a chronic or mild and continued process of invasion and ulceration is less known than the comparable condition of chronic intestinal amebiasis although when symptoms of progressive disease are present, they may be more pronounced. Not uncommonly there is a chronic and intractable diarrhea, and anemia and emaciation may be conspicuous.

In the treatment of balantidiosis stovarsol has been reported as efficacious in destruction of the organisms in the intestine. Benzyl benzoate has also been reported to be useful. Bismuth subnitrate is used to relieve cramps and diarrhea. Colonic irrigations of concentrated solutions of quinine have been reported to relieve the symptoms of balantidial dysentery and to reduce the number of parasites, even when not effecting a radical cure. The reduction of carbohydrates, the use of a milk and egg diet during the treatment of dysentery, and the use of fresh fruits and vegetables are dietary measures of importance.

As in the case of intestinal amebiasis the treatment of this condition consists of measures aimed to destroy the parasites, to aid in healing of ulceration, and to overcome constitutional effects. It should be borne in mind that a cure is not obtained unless the organisms are eliminated.

COCCIDIA

Although coccidia are protozoa which may be parasitic in the intestine of man, the infection is so rare in most countries, including the United States, that little attention will be given it here.

No definite clinical state is associated with coccidiosis in man, the host being usually in apparent good health, although the organism passes a part of its life cycle in the epithelium of the intestine, presumably the small bowel. It is indicated that there may be a temporary diarrhea and abdominal discomfort but that the infection is self-limited and that spontaneous recovery is complete. The species infecting man is *Isospora belli*, usually erroneously called *Isospora hominis*.

BACTERIAL MUTATIONS*

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SEVERAL investigators within the past few years have studied the variations which strains of bacteria may present in the course of their cultivation on artificial media. Their results have been accepted by some and denied by others. If both conceptions are correct one may conclude, first, that bacterial variation is only an occasional phenomenon—some strains may vary, but the majority do not—and second, that since variation occurs only with certain strains the cause of this phenomenon is foreign to the bacterium. When it is present variation occurs, when absent the bacterial culture remains stable.

Variations which occur among living beings may result, in general, from three different phenomena

1 The existence of a life cycle such as one finds in the case of protozoa and insects. This phenomenon is easy to recognize for it is cyclic, the variations are "ordinate". All the characters vary together as a whole. Moreover, in a species which has a life cycle, all the individuals suffer the same variation when placed in identical environments.

2 The adaptation to new external conditions. This phenomenon is not cyclic. One can produce easily such variations with bacteria when they are cultivated in what one might term "abnormal" media, but generally such variations are transitory. The character "sporulation" is the only one, we think, that undergoes a permanent modification after a strain has been cultivated or exposed to the presence of certain antiseptics.

3 Adaptation to new internal conditions. New internal conditions always result from the subjection of an organism to a parasite. Such parasitism is termed "symbiosis" when the host adapts itself to this new condition. This adaptation is always correlative to modifications of the characters of the host (as well as modifications of the characters of the parasite). The mutations which develop due to symbiotic relationships have been studied very extensively by botanists during the last fifty years, especially since the paramount studies of Noel Bernard¹. As far as variation is concerned, bacteriology is a division of botany, and it is remarkable how very few bacteriologists know anything about the researches dealing with variations done by botanists.

To which of these phenomena is the variability of bacteria related? In other words, which one is the cause of the variations?

It appeared to the senior author, from experiments carried on since 1920,² that the best manner to get at the cause of bacterial variations was to take a nonmutating strain and infect it with bacteriophage, in other words to produce an experimental "bacterium bacteriophage" symbiosis, and to observe what takes place when a culture subjected to such an infection is studied continually over a period of years.

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Our studies have been concerned with the *Salmonella enteritidis* because of its pathogenicity for the mouse. Variations of the character "virulence" is certainly one of the most important. In fact, from the "man" point of view the most important. The original strain was strain 904 obtained from the American type collection culture, isolated in 1924 from the commercial rat "Danysz virus". At first we made a series of purifications by isolated colony study. Preliminary tests were performed in order to find out whether our strain was free from bacteriophage. Repeated experiments failed to show the presence of a phage. During the last two years we have used all the known methods to try and extract a phage from this strain but without success. More than 300 transfers of that strain have been made on various kinds of media, including those described by Hadley, but in no case have we observed the least variation, its characters have remained stable.

In December, 1929, we produced the lysis of a culture of that stable strain of *Salmonella enteritidis* by adding to the culture 0.01 ml. of a bacteriophage originally isolated from the stools of a convalescent typhoid patient, and since that time cultivated at the expense of *Escherichia typhi*. The lysed culture remained clear for eight days and then a slight secondary growth appeared. On the fifteenth day after the lysis appeared infusion plates and Endo agar plates were inoculated by spreading over their surfaces drops of the secondary culture. The colonies which were macroscopically different from those of the pure *Salmonella* were inoculated into infusion broth, from these cultures, new isolations were made in infusion agar and cultures derived from colonies which were completely resistant to the phage were kept for future experiments. In this manner we obtained 21 different strains. We could have selected more strains for every one of the 21 strains have continued to present new variants from time to time but had we done so our study would have become too complicated. In fact we could not study completely more than eight strains, the first eight isolated.

For lack of time we cannot describe in detail the aspect of these cultures. We shall merely state that the colonies of Mutant I are smooth, small (1 mm after twenty-four hours) and refractive, colonies of Mutant III are large (3 to 4 mm), opaque and as mucoid as those of the Friedlander bacillus. Mutant I cultured in infusion broth causes a slight homogeneous turbidity, Mutant II, on the other hand, in the same medium shows no turbidity, the broth remains clear, the culture forming clumps in the bottom of the tube. Mutant IV cultured in the above mentioned medium develops a marked turbidity. Every one of the eight mutants is different.

Immediately after isolation all of the mutants were entirely resistant to the action of the original bacteriophage. However, filtrates from these cultures contained a phage active against the original *Salmonella*. Hence, every mutant was a carrier of bacteriophage.

After 10 subcultures (in broth and on agar) Mutants II, IV, V, and VII continued to carry the phage but at this time were sensitive to the original phage. Mutants I, III, VI, and VIII also carried the bacteriophage, but were resistant to the original phage.

After 150 subcultures the eight mutants were sensitive to the original phage. Also the presence of phage in broth culture filtrates of Strains I, III, V and VI could be easily recognized for these filtrates produced lysis of the primitive *Salmonella*.

A series of experiments were carried out to determine whether Mutants II, IV, VII, and VIII were free from phage. Several isolated colonies from each of the mutants were carefully studied in a number of preliminary experiments. The results of the main series of experiments demonstrated (1) that from one of six colonies isolated from Mutant II, we could show the presence of a phage active against the original *Salmonella*, (2) that from thirty colonies picked from Mutant IV only one could be shown to be a phage carrier, (3) that from 100 colonies picked from Mutants VII and VIII not one colony could be proved to carry bacteriophage active against the primitive strain.

It seems therefore, that certain colonies are carriers of bacteriophage while others are free, but further investigation proved that this was not the case. Actually in colonies which appear to be free from phage the phage is "latent." This was true not only in the colonies isolated from Mutants II and IV but also in I, III, V, and VI.

This may be demonstrated in the following manner. A drop of culture of Mutant III is spread over the surface of an infusion agar plate, from the resulting growth a Friedlander-like colony was isolated. This colony was cultured in broth and the resulting growth passed through a filter. This filtrate possessed no action against the original *Salmonella*. A drop of this broth culture (before filtering) was spread over the surface of an agar plate. After incubation the majority of colonies were Friedlander-like. Interspersed among the colonies were a few nonmucoid colonies and some tiny colonies. Two colonies of each of the three types were inoculated into broth and after a twenty-four-hour incubation period were filtered. The filtrate from one of the Friedlander-like colonies and from one of the nonmucoid type colonies produced lysis of the original *Salmonella*. The filtrates of the other four colonies were negative.

We conclude, therefore, that in these mutations all of the colonies are phage carriers, but that in some of them the phage is active, in others it is "latent." The number of colonies (that is to say the number of bacteria) which carry an active phage varies according to the mutant. In our series with Mutant VI approximately every one out of two individuals carries an active phage, with Mutant IV only one out of thirty. The rate of phage reproduction is different also. It is rapid in the case of Mutant VI, very slow in Mutant IV. Thus it appears that in the case of Mutant VI there is a rapid reproduction of the phage at the expense of numerous bacteria, while few absorb it, then many phage corpuscles are free in the liquid and pass in the filtrate, on the contrary, in Mutant IV, a few bacteria reproduce the phage and many absorb it, with a result that no corpuscles are free and found in the filtrate.

But there is another phenomenon which conceals bacteriophage.

We could never detect the presence of a phage acting upon the primitive *Salmonella* in Mutants VII and VIII. However the action of filtrates of these mutants were tested against cultures of the other mutants, and it was found that Mutant VII contained a phage acting only upon Mutant II.

A broth culture of Mutant VIII was streaked on an agar plate and 20 colonies picked for study. Broth cultures of these colonies were filtered, and the filtrates tested upon cultures of the other mutants, as well as upon the original culture. Only one was active and this one lysed a culture of Mutant VII. It appeared that the bacteriophage had lost its pathogenicity for the primitive strain but retained its virulence for certain mutants.

All of these mutants continued to be carriers of phage after 150 subcultures, and still persisted in showing "dissociations." As an example of this let us consider for a moment Mutant III. This particular organism produced typical Friedlander-like colonies on agar plates, and even after 50 subcultures there was no change noticed in these colonies. After 150 subcultures we did observe again "dissociations," 90 per cent of the colonies were opaque, Friedlander-like, 2 per cent were transparent, Friedlander-like, 3 per cent were large, non-Friedlander, and 5 per cent were tiny colonies.

In fact "dissociations" are continual in strains which are bacteria bacteriophage symbiosis in contrast with the original bacteriophage-free *Salmonella* which does not vary. The phenomenon is not a simple one, and under natural conditions, it must be still more complicated, since in nature, variations take place not because of the action of one strain of phage (as in our experiments), but because of the action of several strains, for in nature bacteriophages are as ubiquitous as bacteria. We have some proof of this, for all of these mutants began to show new dissociations when subjected to the action of a new phage (one isolated from sewage and possessing a powerful action).

AGGLUTINABILITY

An antiserum having a final titer of 1:3200 was prepared by injecting 3 rabbits with the original strain of the *Salmonella*.

Table I gives the results of agglutination tests after 10 subcultures.

TABLE I
AGGLUTINATION OF THE MUTANTS AFTER 10 TRANSFERS

	1/100	1/400	1/800	1/1600	1/3200
<i>Typhi murium</i>	+++	+++	+++	+++	+++
Mutant I	+++	+++	+++	+++	++
II	+	++	+	-	-
III	-	-	-	-	-
IV	-	-	-	-	-
V	+++	++	++	++	+
VI	+++	+++	+++	+++	+++
VII	-	-	-	-	-
VIII	+++	+++	+++	+++	+++
IX	+++	++	++	-	-
X	+++	+++	+++	+++	+++
XII	-	-	-	-	-
XIII	++	+	+	+	-
XIV	+++	++	+	+	-
XV	++	++	+	+	+
XVI	-	-	-	-	-
XVII	+++	+++	+++	++	++
XVIII	+++	+++	+++	+++	++
XIX	+++	+++	+++	+++	+++
XX	+++	++	++	++	++
XXI	+++	++	++	-	-

After 100 subcultures Mutants I, VI, and VIII completely agglutinated at a titer of 1:3200. Mutants IV, III, V, and VII were not agglutinated in a dilution of 1:100. For Mutant II agglutination was only partial from 1:100 up to 1:800.

Adsorption tests showed that the nonagglutinating mutants do not even adsorb the agglutinins (Table II).

TABLE II

ADSORPTION OF AGGLUTININS. SEPLEM ANTITYPHIMURIUM, 2 ADSORPTIONS BY MUTANT

		AGGLUTINATION OF TYPHIMURIUM		
		1/100	1/1000	1/10000
Mutant	III	+++	+++	+
	IV	+++	+++	+
	VII	+++	+++	+
	XII	+++	+++	++
	XVI	+++	+++	++
Control	unadsorbed on Typhimurium	+++	+++	++

FERMENTATION STUDIES

After 10 and 20 subcultures, fermentations are as shown in Table III.

TABLE III

		DEXTROSE	LACTOSE	MANNITE	SUCROSE	DEXTROSE	SALICINE	MALTOSE
Orig	Salmonella	AG	—	AG	—	AG	—	AG
Mutant	I	A	—	A	—	—	—	A
	II	AG	—	AG	—	A	—	AG
	III	AG	—	A	—	—	—	AG
	IV	A	—	A	—	A	—	AG
	V	AG	—	AG	—	AG	—	AG
	VI	A	—	AG	—	AG	—	AG
	VII	A	—	AG	—	AG	—	AG
	VIII	A	—	AG	—	A	—	AG

After 60 and 150 subcultures, fermentative characters of II, VII, and VIII are normal, same as original Salmonella. Mutant III is normal except that it does not produce gas in mannite medium. Fermentative characters of Mutant I have remained abnormal, as they were after 10 subcultures.

VIRULENCE

The virulence of the original strain has been tested repeatedly on mice and has not varied during the course of these experiments. One hundredth milliliter of a twenty-four-hour broth culture administered by mouth to ten mice killed all of them within seven to thirteen days. A dose of 0.001 ml kills 6 to 8 out of ten mice within the same period. The virulence of the mutants has been tested after 10, 100 and 150 transfers.

In the following experiments we have considered our mutants to be non-virulent when ten mice each receiving a 0.1 ml of a twenty-four hour culture by

mouth still survive after a period of thirty days. These mice were then given a 0.01 ml of the original *Salmonella* in order to determine whether their survival was due to a natural resistance. No such natural resistance was ever demonstrated.

After 10 subcultures Mutants I and III are avirulent (all 10 mice survive). Mutants VI and VIII (all 10 mice die) are as virulent as the original strain. Further experiments with Mutant VIII done with doses of 0.001 and 0.0001 ml show that its virulence is about the double that of the virulence of the original *Salmonella*. Mutants II (4 mice die), IV (2 mice die), V (3 mice die) and VII (5 mice die) are all less virulent than the original culture.

After 100 subcultures Mutants I and III are still avirulent and IV and VII are also avirulent. Mutants II (4 mice die) and V (1 mouse dies) are slightly virulent. Mutants VI and VIII tested with doses of 0.01, 0.001, and 0.0001 ml prove that in the case of Mutant VI the virulence has decreased slightly, while the virulence of VIII is still greater than that of the primitive strain.

After 150 subcultures Mutants I, III, and IV are avirulent. II only slightly virulent (1 mouse dies). V and VII weakly virulent (2 and 3 mice die). VIII virulent, but not hypervirulent (8 mice out of 10 die) after infection with 0.01 ml. VI virulence has increased (10 mice out of 10 die) after infection with a dose of 0.001 ml.

The question of what happens to avirulent mutants after they have been ingested by mice next occupied our attention and a series of experiments were done in an attempt to answer this question. A series of ten mice were infected with 0.1 ml of a culture of Mutant I. Thirty days later these mice were sacrificed and their splenic pulp spread over the surface of agar plates. All were bacteria free. When the same procedure was repeated using as a test culture Mutant III, the result was different. Four of the ten spleens examined yielded positive evidence of contamination with Mutant III.

A series of 20 mice received by mouth 0.1 ml of a culture of Mutant III. Forty-two days later one of the mice died and an organism similar to Mutant III was recovered from the heart's blood and spleen of this animal. This organism showed no increase in virulence. Seven of the remaining nineteen died during the summer but no examination was made. On November 10 of this year (seven months after the original infection), a mouse died and from the heart's blood a pure culture of an organism similar to Mutant III was isolated. The remaining eleven were killed seven months after the infective dose was given, cultures of a bacterium similar to Mutant III were obtained from the spleen of four of these mice.

From these experiments we conclude that Mutant I is absolutely avirulent, whereas Mutant III shows some capacity to infect mice. However, in contrast to the original *Salmonella*, which produces an acute disease, Mutant III produces a chronic one.

Mutations brought about by bacterium bacteriophage symbiosis are not only a product of laboratory experimentation, but occur also in nature due to the same cause. Two examples may serve to support this fact. During the course of

this investigation in three batches of mice which we received, one or two mice were sick. Several days later all the remaining animals appeared to be in good condition. When several were sacrificed, however, we isolated from the spleens of these mice mutants contaminated with phage. Their characteristics, including virulence, were similar to those of our Mutants I, V, and VII. Even more striking is the experiment with another strain of *Salmonella*. As is well known, the Pasteur Institute of Paris prepares a virus which is distributed for the destruction of rats and mice. This virus is a culture of *Salmonella enteritidis* (Danysz virus) which came originally from the same source as our primitive *Salmonella*. The latter, however, has been cultivated on artificial media while the Paris strain has undergone many repeated passages through rats and mice in order to exalt its virulence. The strain is now hypervirulent and will cause an acute fatal disease even in wild rats when they ingest this organism. However, the Pasteur Institute strain is not a pure culture, as we have recognized it is a mutant and contains a phage inactive upon our primitive *Salmonella* but strongly active upon our Mutant VII. Apparently the exaltation of the virulence of this Pasteur Institute strain is not the product of animal passage, but the result of a sudden mutation which occurred by chance in the intestinal tract of one of the animals used in the serial passages. The exaltation of virulence by this means is probably very rare in nature, a decrease in virulence very common.

We have also experimental evidence to show that carriers of *certain* of our mutants are protected against ingestions of the primitive virulent *Salmonella*. Twelve mice were fed daily for eight days 0.1 ml. of a culture of Mutant I. They then received by mouth 0.01 ml. of the virulent primitive *Salmonella*. Following this, they again received by mouth 0.1 ml. daily for ten days a culture of Mutant I. Eleven and fourteen days after infection with the original virulent *Salmonella* two mice died. The other ten survived. All six control mice died within eight to thirteen days after infection.

Twelve mice were infected with 0.01 ml. of the virulent *Salmonella* and thereafter received 0.1 ml. of a culture of Mutant I daily for ten days. Three mice succumbed (nine, twelve and eighteen days after the infection). The remaining nine survived. All six controls died.

Similar experiments carried out with Mutant IV did not show any protective action.

In relation to the problem of carriers these experiments show that not all the carriers are infective. Some carry avirulent mutants, others mutants of low virulence, few of them carry mutants whose virulence is equal to that of the primitive strain, and finally some may carry mutants which may be hypervirulent. The latter are very rare in nature. We do not intend to generalize when we state the above, but it is certainly true in the case of mouse typhoid. Moreover the senior author has demonstrated that in cholera, mutants are the principal cause of variations in the virulence of the vibrio but in this case all the mutants are avirulent.³

This investigation shows that the principal cause of variations among bacteria is a result of a symbiosis and certainly not the result of a life cycle. The variations are not cyclic but appear in a very disorderly fashion. What is more

TABLE IV
CHARACTERS OF MUTANTS 150 TRANSFERS

	AGGLUTINABILITY		VIRULENCE	FERMENTATIONS
	1/100	1/800		
Typhi murium	+++		1	Normal
Mutant I	+++		0	Abnormal
II	+		1/100	Normal
III	-		?	Abnormal
IV	-		0	Normal
V	-		1/10	Normal
VI	+++		1	Normal
VII	-		1/10	Normal
VIII	+++		1/2	Normal

characteristic is that each character may vary by itself as an entity, without having any repercussive effect upon the other characters. If we consider for example the three characters "virulence," "agglutinability," and "fermentation," we observe that there is no relation between the variation of one of them and the variation of the others.

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BACTERIOPHAGE IN CLINICAL MEDICINE*

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WHETHER bacteriophage has a place in clinical medicine is a question that remains to be answered. That bacteriophage is in wide use in clinical medicine is a fact which as yet is not generally recognized. In Europe especially in France, England, and Germany, one laboratory alone is distributing in the neighborhood of fifty liters a day. In South America although no figures are available the amount used is considerable. In the United States three biologic concerns are selling this product. In addition a score or more private distributors are furnishing this material for experimental purposes. In Michigan the monthly output of bacteriophage has been about ten liters during the past two years. It is evident to all concerned that despite the entire absence of paid advertising, and in the face of strong opposition in some quarters the number of patients receiving some form of bacteriophage treatment is relatively large and is increasing daily at a rapid rate.

The growing interest in bacteriophage as a therapeutic agent and the increasing demand from both patients and physicians presents a serious problem to those who are interested in the study of this principle. Because of conflicting experimental observations, enthusiastic and poorly controlled clinical application, and rapidly expanding commercial exploitation, a situation is developing which will, unless guided and checked, lead to the ultimate rejection of bacteriophage by all who make any pretense to the practice of scientific medicine. So infinite are the possibilities which the discovery of this principle has made apparent that it would be nothing short of a calamity to have its continued investigation retarded or even halted through the unbridled enthusiasm of those who, without regard to the mechanism of its action, make use of this principle whenever an opportunity presents itself.

Clinical observations without regard to theory have thus far constituted the chief impetus to the increasing use of bacteriophage. The glowing reports of successful treatment of conditions oftentimes regarded as hopeless, are characteristic of the sort of propaganda that has kept many doubtful biologic products in favor. That they have stimulated the use of bacteriophage is unquestionable, and it is equally certain that they should by no means be considered as valueless. Difficult as it is to draw conclusions from data of this type, the very weight of testimony in time becomes impressive.

Since clinical experience has been on the whole favorable and at the same time unconvincing, it appears that but little can be contributed by a detailed presentation of the material found in the literature. The methods have been similar in most instances the results especially in the past few years almost invariably encouraging. On the other hand the theory upon which therapy is based has been repeatedly attacked. The various properties of bacteriophage

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other than lysis have not received adequate attention, and animal experimentation has failed to provide support for clinical expectations. For these reasons it would appear that a presentation of some of the theoretical aspects of the problem would give a more accurate index of the position of bacteriophage in clinical medicine than would the more spectacular but less reliable figures of clinical experiments.

The so-called bacteriophage in general use for therapeutic purposes is in reality a rather complex mixture, one element of which is the bacteriophage proper. Each of the components of the mixture has certain biologic activities, some very well known, others as yet scarcely realized. The lytic principle itself possesses attributes other than lysis, among which may be mentioned ability to transform bacteria, i. e., to instigate bacterial dissociation, and ability to stimulate phagocytosis. The spectacular nature of the phenomenon of lysis has so far, however, overshadowed any other virtues possessed by the bacteriophage or by the other components of the filtrates in which the principle is contained, with the result that therapy has been based almost entirely upon the expectation of *in vivo* lysis. Almost without exception clinical experiment has been planned to provide the most favorable condition for lysis. This has resulted in a selection of cases to be treated and a choice of methods and procedures calculated to favor the lytic phenomenon.

One of the fundamental conditions imposed by the adoption of lysis as a basis for therapeutic application is that the infecting organism be susceptible to lysis *in vitro*. The effect of this concept upon procedure is obvious. The infecting organism must first be isolated. It must then be tested with known bacteriophage to determine whether lysis can occur. In the absence of lysis, an effort must be made to prepare a bacteriophage of maximum activity against this organism. In the event that a satisfactory bacteriophage is discovered, care must be used in the treatment to avoid the formation of antibacteriophage antibodies. Since polyvirulent bacteriophages are not common and since the preparation of specific bacteriophages for given cases is difficult and frequently impossible, the number of patients suitable for treatment is limited. It is not uncommon to find antibacteriophage serum in patients not previously treated with bacteriophage, which fact still further limits the possibilities.

Serious as are these objections to bacteriophage therapy, the failure to demonstrate that *in vivo* lysis can occur under the most favorable conditions has brought about what amounts to a complete collapse of the theory. Here, however, one must be cautious in generalizing, for conditions within tissues and in the circulating blood are not comparable with conditions in the intestine, the urinary bladder, and body cavities. Direct evidence of lysis in the intestine is difficult to obtain. In the isolated loop of intestine, Leitner¹ easily demonstrated bactericidal activity of bacteriophage. If this experiment has not been confirmed, it is equally true that no one has shown that lysis does not occur in the intestine. There is likewise much indirect evidence to the effect that here at least bacteriophagy can be expected. That lysis can and does occur in the urinary bladder has been demonstrated by Maurice² and Laikum.³ It is likewise not improbable that in the absence of extensive purulent exudate lysis can occur in other cavities.

In the circulating blood and within tissues, however, bacteriophage is of doubtful occurrence. Applebaum and MacNeal⁴ have recently demonstrated the inhibitory action of blood and pus. Kruenger and Northrup⁵ have indicated that a concentration of bacteriophage sufficient to cause lysis in blood or tissues is difficult to obtain and is probably never reached in ordinary clinical experience.

In the light of available information, one can only conclude that if the effectiveness of bacteriophage therapy is dependent upon *in vivo* lysis, its application is limited to enteric infections with some slight hope for kidney and bladder conditions. Even then the treatment is limited in application and must await considerable improvement before it can have universal utility.

Clinical experience whatever it lacks in scientific value has, however, clearly indicated the inherent possibilities in bacteriophage therapy in conditions where lysis could scarcely be expected to operate. The treatment of staphylococcus infections, especially of the skin, has been sufficiently encouraging to warrant continued and more exacting trial and if lysis cannot be offered as an explanation for results, there are other and better explanations available.

In the preparation of bacteriophage, bacteria are lysed and as a result of lysis there must remain in the filtrate a certain amount of bacterial protein. The extent to which this protein is hydrolyzed to simpler compounds is not known at present and probably varies considerably according to the bacteriophage and bacterium used and the condition under which lysis occurs. Many investigators, Pache and Urech,⁶ Schultz,⁷ and others, contend that hydrolysis is marked and that very little bacterial protein remains. On the other hand the production of bacterial antibodies by these lysates has been repeatedly observed, notably by Arnold and Weiss,⁸ Arloing, Jossier and Narbonne^{9, 33} and Larkum.¹⁰ Furthermore, I have always found in lysates of staphylococcus that amino nitrogen represented only a small fraction of the total nitrogen present.

The clinical procedure recommended by the Michigan Department of Health and which has been used in more than 2,000 treatments is based upon the assumption that these bacteriophage filtrates are in reality vaccines, although we have not failed to provide a potent bacteriophage along with the proteins. We have always insisted upon inoculations in treatment regardless of the type of infection, and although no conclusions can be drawn as a result of our experience, we have the distinct impression that a very considerable immunity is established after inoculation. The production of antibacteriophagic sera is in our estimation a boggy not to be feared, and as time goes on it appears more and more possible that our doses (2 c c) are much too small. Further support for our beliefs is furnished by Stout,¹¹ who for a number of months has been using large and repeated inoculations with good results. From the same source we learn that bacteriophage prepared from freshly isolated strains of staphylococcus is much more effective than that prepared from old cultures. This checks with our own experience.

Local applications of bacteriophage in treatment of staphylococcus infections have been widely used and have been successful according to Rice,¹² who believes that this method is a combined bacteriophage and antiviral action. If one can attach any significance to the numerous reports of successful application of the principle of local immunity, it would appear that such results as

attend the local use of bacteriophage might be attributable to some such mechanism. In view of our present lack of information on the subject of antiviral, however, it is impossible to offer this as a contributing factor in the results.

Bacteriophage, it is claimed by d'Herelle,¹³ Smith,¹⁴ Arnold and Weiss,¹⁵ and by Nelson,¹⁶ has a remarkable opsonizing action. Bacteria which have had only a brief contact with the lytic principle are much more readily ingested by leucocytes than are normal organisms. With Knudsen, I¹⁷ checked such results, and working with purified bacteriophage, we demonstrated that the bacteriophage itself rather than the proteins brought about the change. Opsonic indices as high as 40 have been reported, although the average for all experiments would be nearer 10. Whether this is significant from the standpoint of therapy has not been demonstrated.

The change in the bacterial substrate through the action of bacteriophage is as yet little appreciated in its therapeutic significance. Bacterial lysis has so far occupied the attention of investigators that the loss of virulence noted in secondary resistant cultures has received little attention. In fact, at first it was believed that those organisms which resisted the action of bacteriophage and developed following lysis had enhanced virulence. This view was held by d'Herelle¹³ who later revised his opinions, and the decreased virulence of such cultures has since been observed by numerous investigators. Hence it is entirely possible that even without lysis bacteriophage may considerably alter the course of an infection.

With so much controversy over the effectiveness of bacteriophage as a therapeutic agent and the mode of its action it would seem that animal experiments might provide an answer, especially since controls could be easily assured. The one great objection to such experiments has been pointed out by d'Herelle¹⁸ and is becoming more generally appreciated by immunologists. Purely artificial infections although they have their place, cannot be accepted as a sound basis for immunologic or therapeutic conclusions. It is essential that experiments be carried on with infections to which the animals concerned are naturally susceptible. Even here artificially induced natural infections do not necessarily reproduce the conditions obtained during naturally transmitted disease. As a result, the situation with respect to controls is almost if not altogether as complicated as with human subjects.

Such experiments as have been conducted with animals have given rather variable results. The early experiments of d'Herelle¹³ with fowl typhoid were not sufficiently extensive to be conclusive. His later work with barbone in buffaloes was apparently satisfactory, although Cowles and Hale¹⁹ make the statement that in a personal communication d'Herelle claims the opposite. In this same paper Cowles and Hale summarize the situation with respect to animal experiments as follows:

"Pyle²⁰ working with an infection by *Bact. pullorum*, and Levy,²¹ Topley, Wilson and Lewis,²² Richet and Hauduroy,²³ and Bionfenbrenner and Koib,²⁴ studying mouse typhoid, all obtained unfavorable results as did Wollman²⁵ with *Bacillus shigae* and *B. danysz*. In experiments on plague in rats Doorenbos²⁶ found that bacteriophage exerted some protective action but Compton²⁷ failed to observe any such effect."

Cowles and Hale, themselves were unable to protect white mice against anthrax by inoculations of bacteriophage and Larimore and Harris²⁸ state that typhoid bacteriophage (in guinea pigs) exerted no prophylactic or therapeutic action on *B. typhosus* infection.

There are, however, a number of animal experiments which tend to demonstrate the relative efficacy of inoculations of bacteriophage as a method of choice in treatment and to attribute the results to the bacterial proteins involved. While Compton was unable to demonstrate any therapeutic value in plague bacteriophage, he was able to immunize rats against infection and concluded that failure to detect bacteriophage in immune animals leads to the conclusion that the immunity is antibacterial rather than protobiotic. Maslakowitz and Kasarnowsky²⁹ immunized rabbits with Shiga bacteriophage and found them protected against ten lethal doses of the bacteria. They, too, present evidence to show that the bacterial proteins rendered available by the bacteriophage provided the protection. Mistral³⁰ was able successfully to treat a paratyphoid infection in hogs by inoculation of bacteriophage and Ailong, Jossierand and Narbonne⁷ could protect guinea pigs against intraperitoneal inoculations of *B. typhosus* when these pigs received first serum from rabbits which had been immunized by inoculations of bacteriophage. This again favors the antibacterial rather than protobiotic action of bacteriophage therapy. Flu³¹ was able to protect rats against 40 lethal doses of plague bacilli by giving preliminary inoculations of bacteriophage. Finally, Lucchini and Villa³² state "There is no evidence yet that bacteriophage itself has been of value therapeutically. The evidence favors the view that such therapeutic results as have been obtained were due to the action of the bacterial protein present."

Any careful reading of the literature must leave one more or less sceptical in his attitude toward bacteriophage therapy. On the other hand clinical experience and the wealth of possibilities inherent in the principle seem to demand the continuation of investigation. But investigation and not mere empirical application is required. While it is probably justifiable in instances where other measures are not usable and where bacteriophage has already given some indication of possible value that the patient should not be denied what may be of assistance, it is equally imperative that the methods of treatment be placed upon a sound scientific basis. It matters considerably whether the bacteriophage or the bacterial proteins are the effective fractions of the filtrates employed, for dosage, method of application, and preparation of the product depend upon an understanding of these facts. Study of the literature does not as yet offer a solution of the problem. It is highly essential that experiments be continued on both animals and human beings with a recognition of all the questions involved.

If several years of investigation into the problem of bacteriophage therapy has contributed anything at all it has been this. That clinically gratifying results in spite of lack of knowledge of the principles involved have been the rule, that ingestion, local application or inoculation of bacteriophage oftentimes in large doses has so far as we can judge failed to complicate the infection or otherwise retard progress toward recovery in the patient, and that above all oppor-

tunity for carefully controlled clinical experiments which apparently can be carried out without detriment or discomfort to the patient is urgently needed

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OBSERVATIONS ON THE BACTERIOPHAGE III*

THE TREATMENT OF COLON BACILLUS INFECTIONS OF THE URINARY TRACT BY
MEANS OF SUBCUTANEOUS AND INTRAVESICLE INJECTIONS OF BACTERIOPHAGE FIL-
TRATES DETAILED CASE REPORTS METHODS FOR PREPARATION OF FILTRATES

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THE accompanying report records the treatment and progress of 46 cases of urinary tract infection with one or more strains of the colon bacillus and one with bacillus typhosus (Case 7). These records are arranged so as to make it easy for the reader to come to his own conclusions. There is much unwarranted enthusiasm concerning the use of the bacteriophage as a general measure in infections of all kinds and in conditions that are secondary to them. Bacteriophage treatment has not yet reached the point where it is unnecessary to individualize. That there is an important place for the bacteriophage in therapeutics cannot be questioned. Its preparation and administration should be under the direction of competent medical men.

Great conservatism should be exercised in order to acquire as much trustworthy information as possible. No field in medicine is so full of variables as clinical medicine, particularly that side which has to do with therapeutics. With work of this character we have a great opportunity for the acquirement of fairly exact information, since technique can be so carefully executed and bacteriologic work so carefully controlled.

There has been no attempt to secure a large number of cases to report. Those that are recorded came in the ordinary run of our Clinic and in private practice. They represent, of course, only a fraction of those that come to the various clinics of the University Hospital. The wish has been that the cases might be studied carefully and for long periods of time. This has been possible in quite a number of them and will be recognized. The records unfortunately are not all as full and complete as they should be. This is due in part to State patients coming to the Hospital with no, or very inadequate, histories, to the necessity of dispensing with the services of special nurses and to the discharge of these cases from the Hospital as soon as possible after the disappearance of symptoms and of the bacilluria. This has made it difficult to secure satisfactory follow-up notes in some cases. Hence it is only possible to say concerning these that sterilization was secured following bacteriophage treatment.

Several problems present themselves for consideration in the treatment of all colon bacillus infections of the urinary tract. One cannot know too much about the history. Have there been previous attacks? Have there been unexplained illnesses in the past, particularly if associated with fever pains in the abdomen and back? Has there been a recent infection, an upper respiratory infection, for example, which may have been a predisposing factor? How long have the present symptoms or manifestations been present? In other words, are

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we dealing with an acute, an acute recurrent, or a chronic infection? One must start out with the knowledge that these different types of infection do not all respond alike to treatment with the bacteriophage. On purely theoretical grounds we may expect a case of recent development to respond more promptly to treatment than a chronic one. In persistent cases a pyelogram should be made. If the case falls in the chronic group a guarded prognosis as to the duration of treatment and of observations should be given, some idea as to the prospects for ultimate sterilization of the urinary tract.

DEVELOPING OR ADAPTING THE BACTERIOPHAGE FILTRATE

Elsewhere we have described in detail the methods employed for the development of filtrates for treatment. After many years of diligent effort it has been impossible to develop a suitable polyvalent stock bacteriophage filtrate which could be recommended or dispensed for treatment of *B. coli* urinary tract infections in general. A potent filtrate for one patient's *B. coli*, which may have taken many weeks to develop, may have no lytic effect whatsoever on an organism isolated from another patient. On the other hand, it may so happen that a filtrate for the second patient may be developed from sewage base or from a stock phage in a few days' time.

All bacteriophage filtrates are polyvalent in the sense that they are lytic for more than one organism, particularly for old laboratory strains. It is always possible to enhance the potency of a bacteriophage from sewage base or from stock filtrates. Among stock filtrates kept on hand, which usually number nine or ten, we often find one that is satisfactory for treatment. If this happens many days' time may be saved.

The greatest problem of all is the adaptation of the bacteriophage to the case strain. The many illustrations of prompt sterilization after an entirely satisfactory bacteriophage has been found, inclines one to feel that no matter how long the infection has been running, if this can be achieved, a satisfactory result will occur. This is well illustrated in the following case.

CASE 30—A W. Bacteriophage adaptation was begun September 16, 1928. A partially potent bacteriophage was not obtained for twenty-four days. It was decided to try it out. After two months' treatment it was of no avail. The patient passed from observation for sixteen months, when she returned for further treatment (March 10, 1930). Because the original bacteriophage proved to be lytic for organisms of several other patients it was kept as a "stock phage." It had been constantly under development all this time, often being fed three times a day with this patient's organism. It was now found to have a very vigorous action against the patient's strain. Nine days after the first subcutaneous injection sterilization was complete. Fourteen subsequent urine cultures over a period of sixteen days were negative. Eleven months later a urine sample showed no cells. There had been no return of symptoms. Unfortunately the intern who saw the patient at this time failed to send a sample of the catheterized specimen to the laboratory.

CASE 29—I R. In this case the development of a satisfactory filtrate took two months' time. It was very potent for the patient's strain. In this patient no attempt was made to treat her with a partially lytic bacteriophage. Three days after the first subcutaneous injection the urine became sterile and continued so for five successive cultures over a period of eight days. There has been no recurrence.

It is true that there is more difficulty in finding a satisfactory bacteriophage for the long-standing cases. On the other hand, one may be found in a comparatively short time, as illustrated in the following case.

CASE 27—D D, 5 years old, had had pyuria and bacilluria for over a year. A very potent filtrate was developed in ten days. The urine became sterile in three days after the first injection and remained so for three successive cultures over a period of four days, when she was discharged. A letter from the mother a month later reports gain in weight, marked general improvement, and absence of symptoms.

Experience teaches that some of these apparently resistant cases will recover if treatment is persisted in, rather if *laboratory efforts* are persisted in, long enough. That the bacteriophage benefits these long standing cases, even though we may be unable to entirely eliminate the organism, is shown by the clearing up of the clinical symptoms and the disappearance of pyuria under its use. In the case (Case 35) of longest duration, probably thirty years, foul urine, cloudiness and excess of cells disappeared.

When the acute cases, those of recent origin that do not give a history of previous attacks, are considered, we find that a satisfactory filtrate is secured in from one to twelve days. In nine it was secured within a week's time. In one case it took nineteen days to develop a suitable bacteriophage. It should be noted that for some unknown reason at this particular time difficulty had been experienced in promptly developing a filtrate for one or two other cases.

Under the influence of treatment with the lytic principle, it is a common observation to see one strain or colony type apparently changed to another. That is a sensitive type changed to a resistant type. In our use of the letters S and R we mean sensitive and resistant. We have not always been able to convince ourselves that all these resistant types produce rough colonies. The impression is easily gained that recurrences are due to the reappearance of the sensitive strain which is often a smooth colony producer. It seems quite probable that the sensitive type may develop from the resistant type which is often a rough colony producer. The greater number of colon bacillus infections of the urinary passages in our experience are of the single type.

Alkalinization—If during the time bacteriophage development is going on the patient is being alkalinized, a certain amount of time is saved. Professor D'Herelle showed quite early in his work that the bacteriophage is not resistant to acid. It thrives best in slightly alkaline media (-6 to -12). "Lysis will not take place in a medium of acid reaction." "The bacteriophage ceases to grow when the medium presents the slightest acidity." Poncher and Cowie have verified these statements. They were able to develop a bacteriophage resistance to temperature above 75° C but not to acidity*. These observations were of course done *in vitro*. Because of this fact alkalinization has been an essential procedure in the treatment of all of our cases. Our routine method at the present time is to put the patient on a basic diet and sufficient sodium bicarbonate alone or together with sodium citrate to render the majority of the urine samples alkaline. If during this treatment the urine occasionally becomes slightly acid to litmus, bacteriophage action seems to continue (examples Cases 24 and 35). In those cases where careful observations were made on the urine reaction, we were convinced of the necessity of this procedure. We have had cases that showed no evidence of bacteriophage action while the urine remained acid, but which did after the urine became neutral or alkaline.

Alkalinization is not always an easy condition to produce unless one sets out to insure its presence. A blanket order for one patient may not fit another. It is best to give the patient pink litmus paper and instruct him how to test his urine. If during the time of phage development the patient is undergoing alkalinization, particularly if it is a long period, and if after the first one or two injections of bacteriophage the urine becomes sterile, one may wonder if the benefit came from the bacteriophage or the alkali. Most physicians have seen cases of B. coli infection of the urinary tract apparently recover on the use of alkali alone, so far at least as the disappearance of symptoms is concerned. This experience is by no means regular. The result following bacteriophage treatment is often so prompt and so marked as to leave no doubt in the observer's mind as to its efficacy. The effect in long standing cases, those that have resisted other methods of treatment including alkalinization, are of particular significance. They offer the greatest proof that the bacteriophage may be effective in the treatment of this type of infection. The almost regular sterilization of acute cases following injection of a bac-

*Unpublished record 1923

terriophage filtrate which we ourselves are entirely satisfied with convinces us that the bacteriophage is responsible. Sterilization in those cases in which the urine reaction has not been entirely satisfactory so far as alkalinity is concerned, where it has been slightly acid, is additional evidence in favor of the bacteriophage being responsible for the sterilization.

Autobacteriophage—Because of the possibility, some might say the probability, of the development of an autobacteriophage in the patient's urine, it is important to utilize the principle of alkalization. One of us has used alkalis in patients for other disturbances for many months at a time in doses commensurate with those we use in bacteriophagy without inducing untoward results. This is particularly true of certain gastrointestinal disturbances associated with hyperchlorhydria. We think no harm will come from this provided a case is a suitable one for alkalization, and the alkali is given at the proper time in relation to the meal. So long as no edema, no unexplained increase in weight, and no breathing disturbance occur, it is safe.

We have not been successful in regularly demonstrating the bacteriophage in the urine of patients with *B. coli* infections either before or after bacteriophage injection. Others have done so, particularly after the injection of bacteriophage. They report that it is an almost invariable finding. We have, however, found three cases in which a bacteriophage had developed in the patient's urinary tract. These have been of great interest. In one (Case 16) it was discovered at the time of the first culture. It occurred in a patient who had been sent to Contagious Hospital because of a suspicion of typhoid fever. Many slowly motile gram negative bacilli, which we believed were *B. coli*, were found in the urine. Blood culture and other typhoid tests were negative. The patient's blood had developed no agglutinins for *B. typhosus*. This boy of eight years, who had been ill for five days preceding entrance to the Hospital, became afebrile by lysis on the third day in the Hospital. The motile bacilli disappeared inside of twenty-four hours after this. They could not be cultivated. The urine contained a bacteriophage that was lytic for several other case strains and for a strain of *B. Coli* isolated from the patient's stool. This illustrates the characteristic manner in which a bacteriophage reaches a high state of potency just before the termination of an infectious process, and suggests that colon infections are naturally terminated this way and that it might be possible to find an active bacteriophage in the urine of such cases should we be able to follow them from day to day as we do a typhoid or a dysentery patient.

In another case (Case 44) which was brought to the Hospital because of a supposed acute appendicitis, and which was referred to this Department eight days later for study, a urine culture taken at this time (Feb. 20, 1931) was found to be loaded with colon Bacilli. Alkalization was begun. Three days later there were definitely fewer organisms in the urine, and an autobacteriophage was discovered. It was still present eight days later. It had little power against the organism it had been living with symbiotically. We endeavored to enhance its virulence artificially and succeeded in doing so to a certain extent. By the sixth fifth day after the first injection of the enhanced autophage, the same organism could still be isolated from the patient's urine. When this culture was left alone, it lysed itself in forty-eight hours, making it impossible for us to keep a strain for further study. The urine was found to be sterile fifteen and twenty days later. No other bacteriophage was used in this case. In another case (Case 43) a bacteriophage appeared in the urine sixty-seven days after the last of four inoculations of bacteriophage filtrate.

Method of Administering the Bacteriophage—There are three methods of administering bacteriophage filtrates, subcutaneous, intravesical, and by direct ureter catheterization. All of these methods may be employed singly or combined as the case may indicate.

Subcutaneous Injection Early experiments of D'Herelle indicate that small doses are definitely more effective than large ones. In his experimental work with Barbone which may be regarded as fundamental, a "solid immunity" could be conferred in forty to sixty days after a single injection of 20 c.c. of the bacteriophage filtrate, in twenty-eight days after 5 c.c., in twenty days after 0.25 c.c., and in four days after the injection of 0.04 c.c. If the animals were inoculated with a culture of Barbone earlier than the intervals indicated for the respective amounts of specific bacteriophage, the animals died. Professor D'Herelle indicated the dose of 2 c.c. for the treatment of colon bacillus infections in man. We have used this amount, and

never more than 3 c.c. subcutaneously. It has been our custom to give the amount on alternating days for three doses.

In our first series we used only the subcutaneous method. There are 10 acute cases which were treated this way. The urine became sterile in 10, 17, 19, 15, 11, 7, 5, 4, 1, and 5 days respectively. There are nine acute cases treated by the combined subcutaneous and intravesical methods, 3 c.c. in the arm, 8 to 10 c.c. instilled into the bladder. The urine became sterile in 8, 2, 2, 6, 2, and 4 days respectively in six, one (Case 16) developed an autophage, one (Case 12) could not return for culture. From this experience it would seem that the combined method is preferable. As the instillation of bacteriophage filtrate into the bladder produces no irritation it seems advisable to recommend this method of treatment for all cases. Should the infection be cystitis alone, this type of treatment might prove more efficacious. We have treated only two cases by means of flushing the kidney pelvis with bacteriophage filtrate. No special advantage was gained over the other methods.

There are four acute recurrent attacks. In three cases treated by subcutaneous injection alone, (Cases 19, 20 and 22) the urine became sterile in 3, 3, 9, and 2 days respectively. One case (Case 21) treated by the combined arm and bladder method became sterile in two days. There are 10 attacks in 8 chronic cases treated by subcutaneous injection alone. The urine became sterile in 13, 3, 9, 3, 2, and 1 days respectively in 6 of these (75 per cent), not at all in 3, and in 11 months in one. There were 19 attacks in 12 cases treated by the combined arm and bladder method. The urine became sterile in 9, 1, 1, 7, 33, 33, 6, 5, 33, 15, 5, and 1 days respectively in 12 of these (63 per cent). In 5 attacks we were unable to sterilize the urine. The average number of days necessary for sterility by the subcutaneous method alone was 6, by the combined method 9. If we exclude the exceptionally long case (Case 35), the average number of days for the combined method was 6.4. Again the combined method seems to be the better.

Bladder Instillation. The patient should present himself for treatment with a full bladder. This insures an adequate sample, and if a man, we think catheterization is easier. He has been instructed to reduce his water intake somewhat before treatment and to refrain from excessive drinking until five or six hours after instillation. The patient should lie down, preferably in bed. He is instructed to hold the urine as long as possible, the idea being to retain the bacteriophage as long as possible. Most people have no trouble in retaining the urine five or six hours. With children it may be more difficult. While this is not an absolute rule, we think it has certain advantages.

Reactions. In our experience, the severity of the reaction is in proportion to the amount of protein the filtrates contain. We have sometimes found that a bacteriophage developed in broth may have a higher potency than that developed on hard media. The latter contains the smaller amount of protein. Its solutions are practically water white. The potency of a bacteriophage may be developed so high in broth as to permit of very high dilution. Such filtrates may not produce marked reactions. These reactions are usually the well known nonspecific protein reactions, such as those that follow the subcutaneous injection of sterile milk or dead typhoid bacilli. If the patient becomes sensitized or has previously been sensitized to any of the proteins contained in the filtrate, a specific reaction may occur. The local and general reactions may be so severe that one may question the advisability of proceeding with the treatment until a more satisfactory filtrate is secured. As a rule, in the larger percentage of patients treated, no uncomfortable reactions occur. With the clear filtrate off solid media only a faint pink local reaction measuring 3 by 3 cm. to 5 by 5 cm. or less, with little or no edema, will be observed. A filtrate that will give a very marked reaction in one patient may have no effect whatsoever in another. No untoward results have followed any of the many hundreds of injections we have given. Children as a rule react less severely than adults.

The question may quite properly arise as to whether the protein content of the filtrate may not be responsible for the disappearance of the colon bacillus from the urine. Indeed we have seen bacteriophage filtrates that have been used in a somewhat promiscuous way in other types of infection which caused us to wonder if some of the effects produced may not have been due to the nonspecific action of the protein. Nonspecific protein therapy has been used

quite extensively in urinary infections Cowie* has given this subject considerable consideration. He found that 3 cc of normal horse serum injected subcutaneously causes the urine in certain cases to become sterile. He had no success with the use of dead typhoid bacillus protein. Because of this fact a special effort has been made to keep the protein content of the bacteriophage filtrates as low as possible. A very convincing experience in favor of the bacteriophage being the responsible factor in addition to its obvious effect on the bacterium in vitro is the fact that sterilization by means of the bacteriophage is the same whether the protein content of the filtrate is high or very low, whether a reaction occurs or not. The case recorded in which a powerful autobacteriophage developed and which terminated in prompt recovery is a very good illustration that the bacteriophage alone was responsible for the cure.

Effect of Repeated Attempts to Sterilize the Urinary Tract with Bacteriophage Filtrate—It has been feared because of the development of bacteriophage antibodies in a patient treated with bacteriophage filtrate that a second series of injections would be of no avail. This is probably due to a misunderstanding of the original experiments on bacteriophage antibodies. D'Herelle† has shown that the bacterium builds up antitoxins against the bacteriophage and by so doing may become more resistant in a given preparation than it formerly was. This occurs if a bacterial suspension is inoculated with a weak bacteriophage. He explains on the same ground the failure of a bacteriophage of the same type to produce lysis when it is massively inoculated into a bacterial suspension. Under this condition the bacteria produce a large amount of antitoxin, whereas if a very small amount of the same bacteriophage filtrate is inoculated into a bacterial suspension which has been highly diluted lysis is complete in twenty-four hours. Because of the high dilution the inhibitory substance has become inactive. This is particularly noticeable when transplants are made on solid media. In the treatment of *B. coli* infections this factor does not enter.

D'Herelle has also shown that antibodies of various types develop in the blood of rabbits following the injection of certain bacteriophage filtrates. It should be remembered that these filtrates contain not only the bacteriophage corpuscles but also the metabolites of the bacteriophage action on the bacteria. Thus there are produced amboceptor, agglutinin and opsonic antibodies against the bacterium and antitoxin and antiferments against the secretory products of the bacteria. In the case of *B. coli* he found bacterial antibodies, agglutinins, for certain strains. These are "usually so weak that their action is almost imperceptible." He is of the opinion that the presence of bacterial antibodies in the blood are of no special significance. The situation is not analogous to that of antibacteriophage Shiga dysentery serum. The Shiga bacillus develops an endotoxin which in turn develops an antitoxin and antibacteriophage antibodies that sensitize the Shiga bacillus and render it more virulent for mice. In this instance an antiummunizing serum is contained in the bacteriophage antiserum. D'Herelle points out that this sensitizing substance, sensibilisme, "develops in the animal only after the second injection," and "but a single minute injection of a culture of antidysentery bacteriophage" is necessary effectively to vaccinate a rabbit against the effects of the toxin.

When this knowledge has been applied to man, it has been found that the injection of sensitized bacteria are of no greater virulence in general than normal living organisms. "They are equivalent." From records of cases contained in this report it will be readily seen that one course of one series of injections of the bacteriophage filtrate does not interfere with a subsequent series producing lysis of *B. coli* in the urinary passages whether given at short or long intervals.

No bacteriophagocidal antibodies develop in an individual after inoculation with bacteriophage filtrate. Mrs. J. O., Case 23, received in all, six injections of filtrate in two series. If phagocidal antibodies develop and are of any significance, one would expect to find them in this patient's serum. With the assistance of Mr. Robert Hicks, various amounts of bacteriophage were subjected to the action of this serum for several hours at 37° C. Even in those tubes that contained only minute amounts of the bacteriophage and a high concentration of serum, no difficulty was encountered in recovering a bacteriophage as vigorous in its action on *B. coli* as it was before being acted upon by the patient's serum.

*The Horse Serum (Foreign Protein) Treatment of Pyelitis and Pyuria. *Am. J. Dis. Child.* 4: 179, 1922.

†D'Herelle. *The Bacteriophage*. Eng. Trans. 1922. Williams & Williams Co.

Effect of Bacteriophage Treatment on the Cell Content of the Urine—Usually the disappearance of pus or increased cells in the urine precedes or occurs simultaneously with the disappearance of B coli. In 38 cases, the excess of cells disappeared before the urine became sterile in 19, simultaneously in 16 and after it became sterile in 3. The disappearance of cells or the subsidence of all symptoms, however, cannot be taken as a criterion for the disappearance of B coli. This will be seen by consulting the records. Unfortunately in some cases notes on the cells are inadequate. This was due to a centering of interest on the bacteriology. A perusal of the cases will convince one that the treatment as carried out has in most cases a definite effect on the pyuria. On the other hand, almost invariably when bacilluria is completely overcome pyuria does not continue.

Discharging the Patient All patients on discharge are requested to continue the alkalinization under the direction of their home physician or the Clinic. Those that are apparently cured are informed that the condition recurs in some cases. They are asked to report if any manifestations occur, and are advised that the earlier they are attended to the easier it is to overcome the infection.

ACUTE COLON BACILLUS INFECTIONS, 18 CASES

The criteria for the diagnosis of acute colon bacillus infections of the urinary tract in addition to the demonstration of the colon bacillus are (1) the recent occurrence that is within a few days to a few weeks, of the well known acute manifestations, (2) the occurrence of manifestations referable to the urinary passages during the course of some other disease, usually an infectious disease, (3) the occurrence of manifestations following a surgical operation, particularly abdominal and pelvic, and following catheterization.

TABLE I

CASE	AGE	SEX	APPROXIMATE DURATION BEFORE TREATMENT WITH BACTERIOPHAGE		URINE BECAME STERILE AFTER BEGINNING TREATMENT IN	NUMBER OF TREATMENTS	URINE REMAINED STERILE FOR AT LEAST*	RETURN OF BACILLURIA AFTER	METHOD OF ADMINISTRATION	DATE OF EXAMINATION
			DAYS	WEEKS						
1	10 m	F		3+	10 days	3	10 days	13 days	Subcutaneously	6-4 25
2	12 yr	F	12		17 days	3	10 days	No return	Subcutaneously	6 13 25
3	4	F	5+		19 days	6	8 days	No return	Subcutaneously	6 15 25
4	27	F	7		15 days	4	5 days	No return	Subcutaneously	6 28 25
5	Ad	F		2+	11 days	4	9 days	No return	Subcutaneously	9 22 25
6	33	F	4+		7 days	3	5 days	No return	Subcutaneously	10 15 25
7		M	Typhoid		5 days	2		No return	Subcutaneously	10 26 25
8**	50	F	3			3		No return	Subcutaneously	6 25 28
9	66	F	6		1 day	3	6 days	No return	Subcutaneously	11 17 28
10	52	F		2+	5 days	3	50 days	No return	Subcutaneously	10 26 29
11	55	F		2+	8 days	8	12 days	No return	Subcutaneously	12 29 30
12**	45	F		2		3		No return	Subcutaneously	2 27 30
13	50	F		2	2 days	4	20 days	No return	Subcutaneously	2 10 31
14	3	F			2 days	3	2 days	No return	Subcutaneously	6 23 31
15	3	F		6	6 days	3	2 days	No return	Subcutaneously	10 2 31
16**	8	M		4	1 day			No return		11 18 31
					Autophage					
17	6	F	7		2 days	3	2 days	No return	Subcutaneously	12 10 31
18	7	F	10		4 days	3	1 day	No return		12 16 31

K—Kidney pelvis lavage B—Bladder

*Last opportunity for a culture

**Could not return for check culture Symptoms disappeared No recurrence to date

***An autobacteriophage was found in urine at first culture Urine sterile following day See record.

There are 12 cases belonging in the first group (Cases 2, 4, 5, 6, 10, 12, 13, 14, 15, 16, 17, 18) (see Table I) The urine became sterile in two to seven days in all but the first three, which required 17, 15 and 11 days respectively Case 12 we assume became sterile The patient has been under regular observation There has been no recurrence to January, 1932 There are three cases in the second group (Cases 1, 3, and 11) The urine became sterile after a longer period, 10, 19, and 8 days respectively In Case 1 there was a reappearance of *B. coli* after thirteen days Twenty-two days after a second course of three subcutaneous injections, the urine remained sterile for thirteen days In the last group there are two cases (Cases 8 and 9) One responded with a negative culture in twenty hours and continued to be negative The other responded with disappearance of symptoms and cells and a distinct diminution in the number of bacteria There was no opportunity for further culturing The patient had no return of symptoms It may be permissible to say this case was at least clinically cured

There are not enough cases in the last two groups to warrant any conclusion as to a difference in time for sterilization to occur The analysis suggests that the earlier the case is placed under treatment the more rapidly it will clear up

CASE 1—N R, aged ten months U H * 10536 On May 14, 1925 the baby was brought to the Hospital because of hydrocephalus, spinal bifida and clubbed feet Two days after

Case 1 Counted as two attacks of bacilluria

DATE 1925	CELLS*	B. COLI	URINE PH	REMARKS
6 4	+	++	6 1 acid	Alkalinization begun
5	++		6 2 acid	
6		+	6 9 acid	3 c.c. bacteriophage filtrate in arm
8		+	7 2 alk	2 c.c. bacteriophage filtrate in arm
10				2 c.c. bacteriophage filtrate in arm
13	0	+	7 1 alk	
16	0	0	7 2 alk	
17	0	0	7 2 alk	
18	+++	0	6 5 acid	
25	0	0		Gaining weight
28	0			
7 7	++	+	6 5 acid	
9	++	+	6 9 acid	3 c.c. bacteriophage filtrate in arm
12		+		2 c.c. bacteriophage filtrate in arm
15				2 c.c. bacteriophage filtrate in arm
31	0	0	7 3 alk	
8 1	0	0	7 4 alk	
8 5	0	0	7 4 alk	
12	0	0	7 4 alk	

*Per low power field uncentrifuged urine

***Estimation of the degree of positiveness* In all of the 1925 observations the cultures were marked + or 0 without any attempt to indicate the numbers of bacilli In subsequent reports we have adopted the following scheme Inoculations are made with a standard 2 mm platinum loop +++ growth completely covering a 3-inch Endos Petri dish in twenty four hours ++ about 200 colonies +- 50 to 75 colonies + about five colonies 0 no growth on plates but an organism can be developed in broth with 3 c.c. of urine 0 no growth in broth with 3 c.c. of urine Many reporters regard a negative Endos Petri dish as evidence of sterility Three cubic centimeters of urine in broth is a much more rigid test Final negative sterility must be on media other than Endos (blood agar or broth) It is of interest to note that if counts are below fifty they are usually about five

*U H, University Hospital C H Cowie Hospital

entering, a temperature of 103.5° developed. Symptoms of an upper respiratory infection were present. On May 22 the urine showed pus and a trace of albumin. On June 4 a urine culture showed B. coli. *Diagnosis*: upper respiratory infection complicated by urinary tract infection, probably pyelitis. Alkalinization was begun. A satisfactory bacteriophage was found in stock. Three subcutaneous treatments on alternate days brought about a termination of the symptoms.

Recurrence of cells and B. coli July 7 indicated the necessity for a second series of injections. The pyuria and bacilluria disappeared in twenty-two days, and continued for thirteen days. No recurrence.

CASE 2—C. V., aged twelve. U. H. 128816. On the morning of June 10, 1925 the patient complained of pain in the right lower abdomen and a feeling of malaise. The following morning she vomited. The temperature was 104°, where it remained until admittance to the Hospital June 13. She vomited several times after entrance. There was no history of a previous attack. There had been frequency and burning micturition. The patient was sent to the Hospital with a diagnosis of acute appendicitis.

Physical examination showed a well developed, well nourished, acutely ill child, definite tenderness on pressure in the right lower quadrant of the abdomen and in the right costo-vertebral angle. Otherwise examination was negative. Leucocytes were 15,300. A catheterized urine sample was turbid and acid, albumin was positive. There were 25 cells per low power uncentrifuged field, some clumps. Culture showed B. coli.

Alkalinization and forced fluids were begun. The temperature reached normal in forty-eight hours, where it remained for several days. The morning of June 21 chill, temperature of 104°, pain in left lower abdomen, and marked tenderness in the left costo-vertebral angle developed. Five thousand cubic centimeters of fluid were given during the following twenty-four hours. The temperature dropped to 101.5°. It will be observed that the so-called sensitive or lytic colonies disappeared from the urine twenty-four hours after the third dose of bacteriophage, the resistant colonies fourteen days after. The patient made a good recovery without recurrence. Detailed record follows.

CASE 2

DATE	CELLS	B. COLI		UPINE P. H.		REMARKS
		P*	L*			
1925						
6 13	++	-	-	5.8 acid	Temp 104°	Alkalinization begun
6 16	+	+	+	7.3 alk	Temp 99°	
6 19	+	-	+	7.3 alk	Temp 98.4°	
6 20	+	+	+	7.2 alk	Temp 100°	
6 21	+	-	-	7.3 alk	Chill, temp 104°, pain 11 abdomen and 10 costo-vertebral angle	
6 22	+	-	+	7.2 alk	3 c.c. bacteriophage filtrate in arm	Temp 104° continued
6 23	+	-	-	7.2 alk		Temp 98.5°
6 24		-	-	7.4 alk	2 c.c. bacteriophage filtrate in arm	
6 25	+	+	0	7.2 alk	24 hours after last injection	
6 26		+	0	7.4 alk	2 c.c. bacteriophage filtrate in arm	
6 28		-	0	7.2 alk		
6 30	+	+	0	6.8 acid	Temperature remained normal. No symptoms	
7 2	+	+	0	6.5 acid		
7 4	+	-	0	6.8 acid		
7 5						
7 7	+	+	0	6.6 acid		
7 9	+	0	0	6.8 acid	14 days after last injection	
7 11	+	0	0	6.8 acid		
7 12	+	0	0	6.8 acid		
7 13	0	0	0	6.8 acid	Temperature normal for 15 days	
7 15	0	0	0	6.9 alk		
7 16	0	0	0	7.2 alk		
7 17	0	0	0	7.1 alk		
7 19	0	0	0	7.1 alk	No recurrence of symptoms	

*P—resistant L—lytic.

CASE 3—D P, aged four U H 131261 This patient was in the Hospital for over a year because of an extensive burn On June 1, 1925 she was sent to Contagious Hospital because of measles On June 15 she was transferred back to the Children's Ward During the routine urine examination, pus and many motile bacilli were found Alkalinization was started A bacteriophage filtrate was ready for injection June 19 Two courses of filtrate were necessary to clear up this case Thirteen days after the last injection the urine became sterile and remained so

CASE 3

DATE	CELLS	B COLI	URINE P H	REMARKS
1925				
6 15	++++	+	6.2 acid	Alkalinization begun
6 16	++++		6.3 acid	
6 17	++++	+	6.3 acid	
6 18	++++	+	6.3 acid	
6 22	++++	+	6.2 acid	
6 23	++++	+	6.3 acid	2 cc bacteriophage filtrate in arm
6 24				2 cc bacteriophage filtrate in arm
6 25				1 cc bacteriophage filtrate in arm
6 28		+		
7 1	++		7.4 alk	
7 2	0		7.4 alk	
7 3	++	+	7.4 alk	3 cc bacteriophage filtrate in arm
7 9	++	+	7.4 alk	3 cc bacteriophage filtrate in arm
7 13	+++		6.9 acid	2 cc bacteriophage filtrate in arm
7 15	+++		6.9 acid	2 cc bacteriophage filtrate in arm
7 16	++++			
7 18	++			
7 22		+	7.1 alk	
7 28	0	0	7.2 alk	
7 30	0	0	7.2 alk	
8 1	0	0	6.9 acid	
8 5	0	0	6.9 acid	

CASE 4—Mrs A Q, aged twenty seven U H 122366 The patient came to the Hospital June 28, 1925 because of pain in the left side and back About one week before entrance she noticed "bladder trouble," as evidenced by marked frequency, nocturia, and burning Urinary frequency became almost constant, and pain became continuous in left side and back However, the pain was worse during certain attacks She never passed blood, no vaginal discharge Temperature rose progressively and her doctor found much albumin and pus in the urine She was given hexylresorcinol treatment Sinusitis of the left nostril three months preceding admission was reported She was ill five weeks Patient was pregnant

CASE 4

DATE	CELLS	B COLI	URINE P H	REMARKS
1925				
6 28	+++	+	5.9 acid	Right ureter B coli +, left 0 Alkalinization begun
6 29		+	5.8 acid	
7 1	++	+	7.4 alk	3 cc filtrate in arm, 25 cc pelvic lavage
7 4	+	+	7.3 alk	By mistake hexylresorcinol was not discontinued June 28, 1925
				2 cc filtrate in arm
7 5	++	+	7.4 alk	
7 8	+++			2 cc filtrate in arm
7 9		+	7.6 alk	
7 10		+	7.6 alk	2 cc filtrate in arm
7 14		+	7.2 alk	
7 15		0	7.2 alk	
7 16		0	7.2 alk	
7 20		0	6.9 acid	Condition has cleared up satisfactorily

Physical examination Temperature 104° Otherwise negative On July 1, 1925 cystoscopic examination was done Both pelvis were irrigated with bacteriophage solution

This patient was given four subcutaneous injections of bacteriophage solution The urine became sterile on the eleventh day after the first dose Whether hexylresorcinol delayed sterilization or not cannot be determined Urinary antiseptics should not be administered during treatment with the bacteriophage

CASE 5—Mrs O'B, aged forty seven C H,* September 22, 1925 The patient complained of painful micturition for over two weeks There were no other symptoms She was otherwise in excellent health The urine showed a marked excess of leucocytes A catheterized specimen confirmed this finding and was positive for B coli Alkalinization was begun and was effected in three days Sterilization occurred in eleven days The patient's symptoms were definitely improved after the second treatment, and had disappeared by the eleventh day There has been no return of symptoms in this patient

CASE 5

DATE	CELLS	B COLI	URINE P H	REMARKS
1925				
9 23	+++	+	5.8 acid	
9 25				3 c.c filtrate in arm
9 26	+++	+	7.6 alk	
9 27				2 c.c filtrate in arm
9 28	+++	-	7.6 alk	
9 29				2 c.c filtrate in arm
10 1	+++	+	7.6 alk	
10 2		-	7.8 alk	2 c.c filtrate in arm
10 3	+	+	7.4 alk	Colonies changing in character
10 6	±	0	7.5 alk	Symptoms have entirely disappeared
10 10	0	0	7.5 alk	
10 15	0	0	7.4 alk	No return of symptoms

CASE 6—Mrs G W I, aged thirty three C H, October 15, 1925 The patient had chronic nephritis with hypertension for two years A few days previous to October 15 she developed urethral irritation A catheterized urine specimen showed 100 to 150 cells per low power field Culture showed B coli type R, large white convex homogeneous colonies and type S, small flat gray translucent colonies In four days we adapted a bacteriophage that was lytic for both types The urine became sterile seven days after the first treatment

CASE 6

DATE	B COLI P S	URINE P H	REMARKS
1925			
10 15	+	-	6.2 acid
10 19			Alkalinization begun
10 20	-	+	7.4 alk
10 21			3 c.c filtrate in arm
10 23	0	+	7.4 alk
10 26	0	0	7.6 alk
10 31	0	0	7.6 alk

CASE 7—Mr L P, aged twenty eight U H 127787 October 26, 1925 The patient entered Contagious Hospital October 12, 1925 with typhoid fever The course of the disease was uneventful The urine culture continued to be positive for B typhosus preventing his discharge Cultures on October 26, 28, 30, and November 4, 6, 8, and 10 were positive Urine alkalinization was effected, and 3 c.c of bacteriophage filtrate were injected sub

*C H Cowie Hospital U H University Hospital

cutaneously on November 6 and 8. On November 10 the urine culture was still positive. On November 11 culture was negative. There was no recurrence. This case is included in this group because of its general interest and the colon typhoid group of bacteria being so closely related with regard to the action of the intestinal bacteriophage.

CASE 8—Miss G. Y., aged fifty. C. H. June 25, 1928. Cholecystectomy with drainage for cholelithiasis and acute cholecystitis.

June 27, 1928. Urine specimen showed sugar and three or four casts per field. Later in the day the sugar was negative, but the specimen was loaded with casts.

June 29, 1928. Sugar negative, no casts, marked excess of leucocytes.

July 4, 1928. Patient complained of irritation and frequency. Catheterized specimen showed cells +++ and many bacteria.

CASE 8

DATE	CELLS	B. COLI	REMARKS
1928			
7 17	+++		Alkalinization begun
7 18	++++	++++	Large colonies
7 23		++++	3 cc filtrate in arm
		±	1 hour after treatment
7 24	±	++++	3 cc filtrate in arm
7 25		++	Tiny colonies. No phage in urine
7 26	±	++	3 cc filtrate in arm. No phage in urine
7 27	0	++	Symptoms have disappeared. No phage found in urine.
			Colonies remain small type

Patient was discharged symptomless but still showing B. coli. She had no further trouble.

CASE 9—Mrs. L. P. H., aged sixty-six. C. H. September 21, 1928. Cholecystectomy for cholelithiasis and cholecystitis, following a sudden attack of abdominal pain, fever, leucocytosis of 20,000, and evidence of empyema of the gall bladder on July 13, 1928. This infection was of over two weeks' duration. On November 7, 1928, forty-seven days after operation, the patient complained of frequency and urethral irritation. Pyuria developed. Urine culture showed +++ B. coli. Alkalinization was begun.

CASE 9

DATE	CELLS	B. COLI	REMARKS
1928			
11 7	++++	++++	Alkalinization begun
11 13	++++	++++	3 cc filtrate in arm
11 14	0	0	20 hours after treatment
11 15	0		3 cc filtrate in arm
11 17	0	0	3 cc filtrate in arm
11 19	0	0	No phage in urine

The symptoms disappeared the day after the first treatment, and the urine became sterile in twenty hours.

CASE 10—Mrs. V. C. W., aged fifty-two. C. H. May 16, 1929. The patient suddenly developed marked hematuria on May 15, 1929 without any apparent cause. She had been in excellent health. There was no history of a recent infection. Physical examination was negative. The first morning urine sample was dark red. There had been no pain on urination. Blood pressure 160/85. Nonprotein nitrogen 42, urine concentration normal, albumin ++, leucocytes and red cells ++. No casts. Alkalinization was begun. A satisfactory bacteriophage was developed in three days. The urine became sterile in five days, and free of organic elements. This patient had no subjective symptoms. There has been no recurrence.

CASE 10

DATE	CELLS	B COLI	REMARKS
1929			
5 16	—		Urine bloody
5 17	—		Urine beginning to clear of blood Alkalinization begun
5 18	—		Urine clear
5 24	—		No casts
5 27	—	—	Bacteriophage adaptation begun
5 30	—		No casts
5 31			3 cc filtrate in arm
6 1		—	Small type B coli No phage demonstrated in urine
6 2			3 cc filtrate in arm
6 3		—	
6 4			3 cc filtrate in arm
6 5	0	0	
6 6			Discharged Urine clear
6 10	0	0	
6 17	0	0	
6 25	0	0	
7 12	0		
7 30	0	0	

CASE 11—Mrs V, aged fifty five C H January 13, 1930 The day after Christmas the patient developed an upper respiratory infection She was in bed five days Had chills all this time Five days later, on the tenth day, she developed urinary frequency, irritation, and pain on micturition She had a similar attack six years previous following an attack of influenza The urine on January 13 showed — cells, many large clumps, and — B coli Alkalinization begun

CASE 11

DATE	CELLS	B COLI	REMARKS
1930			
1 13	—	—	
1 14			3 cc filtrate in arm, 10 cc in bladder
1 16	—	±	3 cc filtrate in arm 10 cc in bladder
1 18	0	—	3 cc filtrate in arm, 10 cc in bladder
1 20	±	—	3 cc filtrate in arm 10 cc in bladder
1 22	0	0	3 cc filtrate in arm, 10 cc in bladder
1 27	0	0	3 cc filtrate in arm, 10 cc in bladder
2 4	0	0	3 cc filtrate in arm, 10 cc in bladder
2 11	0	0	3 cc filtrate in arm, 10 cc in bladder
11 3	0	0	

A bacteriophage was adapted for the patient's organism in twenty four hours by feeding it three times a day with the patient's organism The urinary symptoms disappeared very quickly, and the urine became sterile eight days after the first injection

CASE 12—Mrs D, aged forty five C H February 27, 1930 The patient had recently been having bladder irritation, frequency, and burning micturition She was a robust, un

CASE 12

DATE	CELLS	B COLI	REMARKS
1930			
3 6	—	—	3 cc stock filtrate in arm 10 cc in bladder
3 8	±	—	3 cc stock filtrate in arm, 10 cc in bladder
3 9	—	—	
3 10	0	—	3 cc stock filtrate in arm 10 cc in bladder

usually healthy individual. These symptoms were preceded some days previous by a severe leucorrhoea, that developed on a long automobile trip, which made normal bladder emptying impossible. A catheterized specimen showed a few cells and +++ B. coli. A suitable bacteriophage was not developed until the 8th day. During this time the urine became alkaline on the usual treatment.

The patient's symptoms were entirely relieved by the three treatments, and the excess of cells disappeared from the urine. It was not possible to make further cultures. There has been no recurrence to date. At least a clinical cure was effected in this patient.

CASE 13—Mrs. P. B. S., aged fifty. C. H. February 10, 1931. Two weeks ago the patient began to have smarting and frequent urination. A sample of urine was said to have shown pus. The bladder was irrigated twice. This caused much pain. The past three or four days she has complained of general aching of the body, and a feeling of heat and chilliness. Gave no history of fever.

General examination aside from the urinary findings was negative. A catheterized urine specimen showed 10 to 15 cells per low power field, uncentrifuged urine, a slight excess of epithelial cells, no casts or red cells. She had been drinking large amounts of water. Urine culture on this date showed B. coli ++. Alkalinization was begun.

A satisfactory filtrate was developed from sewage base in ten days. It will be observed from the clinical record that no change had been effected during the first thirteen days except that the complaints had been improved. Two days after the first bacteriophage treatment the urine became cell and bacteria free. No recurrence to date.

CASE 13

DATE	CELLS	B. COLI	CLINICAL FINDINGS
1931			
2 10	+	++	Alkalinization begun
2 23	+	+++	Symptoms better. 3 cc filtrate in arm, 10 cc in bladder
2 25	0	0	Symptoms disappeared. 3 cc filtrate in arm, 10 cc in bladder
2 27	0	0	No symptoms. 3 cc filtrate in arm, 10 cc in bladder
3 4	0	0	3 cc filtrate in arm, 10 cc in bladder
3 17	0	0	Continues to be symptom free. Urine alkaline. Discharged. No recurrence to date.

CASE 14—M. G., aged three. U. H. 267842. June 23, 1931. Patient sent to Hospital because of a heart murmur, unconscious attacks associated with clonic spasms, and pus in the urine on several occasions.

Examination. Well nourished girl. Temperature 99°. No pathologic findings. Heart has been entirely negative. Kahn negative. Urine showed 500 cells per low power field. Eyes and fundi negative. Skull x-ray negative.

Diagnosis. Epilepsy, colon infection of genitourinary tract. This patient was not on a ketogenic diet.

CASE 14

DATE	CFLLS	B. COLI	REACTION	CLINICAL NOTES
1931				
6 25	++	+++	acid	Alkalinization begun
7 2	++	+++	acid	
7 15	++	+++	alk	3 cc filtrate in arm, 7 cc in bladder
7 16				Urine clear
7 17	0	0	neut	3 cc filtrate in arm, 7 cc in bladder
7 19	0	0	alk	3 cc filtrate in arm, 7 cc in bladder
7 24				Discharged. Urine clear. Return in six weeks.

CASE 15—D S, aged nine U H 275513 October 2, 1931 Patient came in with a history of attacks of fever beginning six weeks ago, accompanied by painful micturition, small clots of blood in the urine, costovertebral pain on both sides, especially on the right, loss of weight and headaches The diet had been restricted as a remedial measure The first attack lasted four or five days The attacks recurred every four or five days After the first attack they lasted from one to three days The mother observed that these differed from the first attack in that with the subsidence of the attacks the urine became cloudy, while during the attacks it was clear She has been confined to bed all this time

Physical examination Poorly nourished child A small round smooth firm movable mass in the abdomen beneath the right costal margin is plainly felt, probably a kidney She has complained of pain in this area The lower pole of the left kidney is just palpable Otherwise examination is negative Temperature 99° to 100°, pulse 80 to 105, respirations 20 to 25

Urine Acid trace of albumin, loaded with leucocytes and motile bacteria, a few red cells, no casts Culture started October 2 showed B coli +++ Blood hemoglobin 55 per cent, red cells 2 500,000, white cells 8,450, nonprotein nitrogen 31 Kahn negative

Skidán rxn showed no evidence of organic change in genitourinary tract

A satisfactory bacteriophage filtrate was developed in ten days The urine became sterile on the fifth day after beginning treatment It should be observed that this patient's urine showed marked improvement as indicated by a marked decrease in the number of cells and bacteria under the influence of alkalization before the bacteriophage filtrate injections were given It was not possible to keep the patient under observation longer When last heard from there had been no return of the symptoms

CASE 15

DATE	CELLS	B COLI	CLINICAL RECORD
1931			
10 2	+		Alkalinization begun
10 3	+		
10 7	±	—	
10 14	+++		
10 15	—	+++	Urine alkaline
10 17	—	+++	
10 22	0	±	3 cc filtrate in arm, 7 cc in bladder
10 24	±	±	Morning sample p.v. 3 cc filtrate in arm, 7 cc in bladder
10 26	±	±	Urine continues to be alkaline
10 27	0	±	Urine alkaline 3 cc filtrate in arm, 7 cc in bladder
10 28	0	0	Discharged on sodium bicarbonate
12 10	0	0	Patient has remained perfectly well

One may question whether the alkalization alone or the bacteriophage was responsible for the change Our experience leads us to believe it was the bacteriophage

CASE 16—E C, aged eight U H 278667 November 18, 1931 Patient entered Contagious Hospital with a history of malaise which had been present for five days A month before entrance he had had a diarrhea for a period of three weeks He recovered from this and returned to school Three days before admittance he complained of headache He drank from a well of questionable water six weeks ago His appetite failed, and he had an attack suggestive of a convulsion followed by coma November 17 He vomited once and complained of pain in the abdomen

November 18, 1931 Temperature 104°, pulse 132, respirations 25 Leucocytes 16,700 Urine showed 4 to 8 leucocytes per low power field a few red cells, an occasional cast, many epithelial cells, and many slowly motile bacilli which were gram negative Diazo reaction on the urine negative Spinal fluid 3 to 5 cells colloidal gold, mastix and Kahn negative Blood serum Kahn negative Blood culture negative

This patient made an uneventful recovery. His temperature, pulse, and respirations returned to normal by 1 p.m. on the third day, and the motile bacilli disappeared from his urine inside of twenty-four hours, leading us to believe the case to be one of acute pyelitis.

Bacteriology. The bacteriology of this case is of considerable interest. The etherized specimen showed many slowly motile bacilli. The sediment was crowded with them. On staining they were all gram negative, characteristic of *B. coli*. The centrifuged sediment was 1½ inches deep. The following morning no bacilli could be demonstrated.

The uncentrifuged urine and the sediment were cultured on plain broth, blood agar and Endos medium. The media had all been freshly prepared the day before. These preparations were placed in the incubator in the evening. The following morning there was no growth on the plates or in the broth. They remained negative for five days, at which time they were discarded.

After the above cultures were started, samples of the same urine and urine sediment were left at room temperature for twelve hours. We were interested to find that no organisms could be demonstrated in either specimen. Their previous presence had been checked by three of us.

We then tested the lytic power of the urine and sediment against four strains of *B. coli*, including a mixed colon culture from the patient's stool. These were planted on plain agar, dried, and carefully washed with urine, leaving control areas around the edges of the Petri dish. No growth occurred after three days. No secondary colonies have since developed. The control areas showed abundant growth.

November 24, 1931. Six days after entrance the urine of November 18, 1931, which had been kept in the refrigerator, was still clear. No organisms could be demonstrated. On November 18 and 19 an active bacteriophage was demonstrated in this urine.

Comments.—It would seem that this experience demonstrates very beautifully the phenomenon, shown by Professor D'Herelle, of the appearance of a bacteriophage of high potency just before the termination of the infectious process. The patient made a perfect recovery from this illness. Had we been one day later in making our observations a satisfactory diagnosis of the case could not have been made. It suggests the advisability of performing urine lytic tests on suspected cases in which a negative urine culture is obtained.

CASE 17.—R. M. K., aged six. U. H. 279952. December 10, 1931. The patient has complained of pain in the lower right abdomen for one week. Nausea and vomiting, fever and drowsiness occurred on the second day. Since this time, the pain has been irregular in its occurrence. With each recurrence of pain she vomits. No vomiting on day of admittance to Hospital. There was slight abdominal pain, no urinary symptoms. Temperature 101.3°, pulse 120. Patient looked acutely ill, cheeks were flushed. There was slightly increased muscle tenderness right lower abdomen, and some tenderness on pressure. No mass felt. Rectal examination negative. Patient cried when rectum was pressed anteriorly in the midline. Otherwise examination entirely negative. Leucocytes 16,000. Urine showed pus and many motile bacilli. Culture done December 10, 1931 showed +++ *B. coli*. When informed of the diagnosis, the mother told us she had had "pyelitis" before. A stock bacteriophage filtrate was found to be very potent for the patient's strain. The urine became sterile within forty-eight hours after the first treatment, and has remained so. Alkalinization was begun on December 11, 1931. Abdominal pain and tenderness disappeared on the second day in the Hospital.

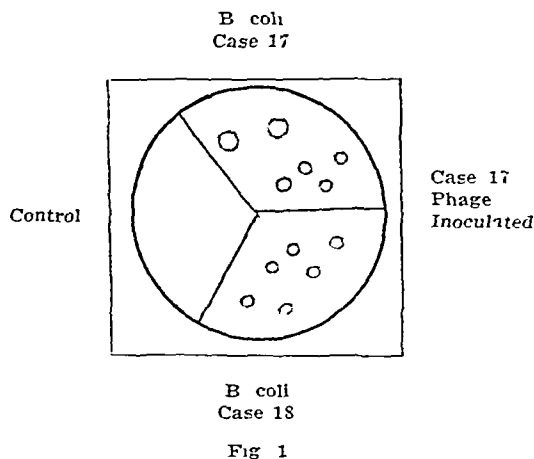
CASE 17

DATE	CELLS	B. COLI	REMARKS
1931			
12 12	+++	+++	Urine neutral. Stock bacteriophage developed potency in 5 days
12 17	+++	+++	Urine alkaline. 3 cc filtrate in arm, 8 cc in bladder
12 19	0	0	Urine alkaline. 3 cc filtrate in arm, 8 cc in bladder
12 21	0	0	Urine alkaline. 3 cc filtrate in arm, 8 cc in bladder

After the first injection the temperature reached 103° and the arm was sore. After the second injection the temperature was 102° . The arm was more swollen and painful. After the third injection the temperature reached 104° in four hours. The arm was not sore.

Bacteriology—One of our stock bacteriophage filtrates was found to be very potent for this patient's strain of *B. coli*. It contained two plaques. The base for the small plaque bacteriophage was sewer water. It had been developed to a high potency against Δ B C *B. coli* (Case 35). This was added to another stock bacteriophage secured from the urine of I H (Case 16) (an autophage), which was made up of large plaques. We were never able to demonstrate small plaques in this patient's urine. In this mixed bacteriophage filtrate the large plaque was very active against the patient's strain. The small plaques had little or no activity. (See notes on Case 18.)

CASE 18—B P, aged seven. U H December 16, 1931. Patient complained of pain in the "right hip" December 6. There had been fever, but no nausea, vomiting or chills, and no urinary symptoms. Temperature on day of admittance was normal, pulse normal, appetite fair. The pain was located in the abdomen just above the right anterior superior spine. There was no costovertebral pain or tenderness. There was a slight hemoglobinemia. The ordinary urine specimen showed pus and bacteria. Otherwise the examination was negative.



The same bacteriophage as that used in Case 17 was very potent for this patient's *B. coli*. The large plaque bacteriophage of this filtrate had no action whatsoever, the small plaque was very active. This is a matter of interest in bacteriophage development and adaptation. This information is secured in the following manner. A Petri dish is divided into three sections as shown in Fig 1. On the upper section *B. coli* from Case 17 urine is seeded. On the lower section *B. coli* from Case 18 urine is seeded, each with a nine hour broth suspension of the respective strain. After drying in the incubator for three quarters of an hour one or two drops of Case 17 bacteriophage filtrate is spread over the surface of the two areas avoiding contamination of one area with the other. The plate is now returned to the incubator for nine hours, or for overnight. At the end of this time it is examined for bacteriophage plaques. The upper section shows both large and small plaques—around the large plaque there is marked haziness while the plaque itself remains clear. Lysis is going on actively about its periphery. The small plaque is clean cut and clear without any haziness or lysis extending beyond its periphery. The lower section, which has been inoculated with the same bacteriophage filtrate, shows no large plaques while the small plaques are present in large numbers. They show no haziness. From this finding we are led to believe that the small plaque is the bacteriophage that is specific for this patient's (Case 18) *B. coli*. The left hand section of the Petri dish is seeded with a third patient's strain and Case 17 bacteriophage filtrate is inoculated onto it. This serves as a control. No lysis occurs.

It will be seen that in developing the filtrate against Case 17 B coli both types of plaques can be demonstrated, but against the patient's (Case 18) B coli only the small plaque is active. Against control B coli neither plaque is active. By carefully feeding the filtrate we finally enhanced the activity of the small plaque so that it became very numerous and very potent for Case 17 B coli, and the large plaque very potent for Case 18.

Another observation of interest that shows the specificity of the bacteriophage was secured by taking a loop of nine hour broth suspension that was lysed by the large plaque and not by the small plaque, and thoroughly mixing it with a loop of nine hour broth suspension that lysed by the small plaque and not by the large, and seeding the mixture on agar in the usual manner. The bacteriophage filtrate (composed of both plaques) was then spread over the seeding. After nine hours' incubation the plate was studded with large and small plaques each acting on its specific organism.

CASE 18

DATE	CELLS	B. COLI	REMARKS
1931			
12 17	+	+++	Urine alkaline. Alkalinization begun 12 16 31
12 18			Slight costovertebral tenderness
12 19	±	+++	Urine alkaline 3 cc filtrate in arm, 14 cc in bladder
12 21	0	±	Urine alkaline 3 cc filtrate in arm, 14 cc in bladder
12 22			Clinical symptoms improving
12 23	0	0	Urine alkaline 3 cc filtrate in arm, 14 cc in bladder
12 25	0	+	Urine alkaline
1932			
1 1	±	+	Urine alkaline 2 cc filtrate in arm, 8 cc in bladder
1 3	0	±	Urine alkaline 2 cc filtrate in arm, 8 cc in bladder. No growth on agar
1 27	0	+++R	Urine alkaline 3 cc in arm, 8 cc in bladder. <i>Very Potent Phage</i>
1 29	0	0	Urine alkaline
3 19	0	0	Urine alkaline 3 cc filtrate in arm 8 cc in bladder
3 21	0	0	Urine alkaline 3 cc filtrate in arm 8 cc in bladder

ACUTE RECURRING COLON BACILLUS INFECTIONS

In this group (Table II) it will be observed that the bacteriophage acts in the same way as it does in the acute cases that occurred for the first time so far as we were able to judge. The sterilization of the urine was very prompt with the exception of the fourth case, which may have required nine days for sterilization. In a subsequent attack in this patient occurring seven months later sterilization occurred promptly as it did in the other cases. It required only two days. The bacteriophage used at this time was very much more potent than it was in the first attack. In the column marked "Return of bacilluria" we, of course, mean that so far as we know there has been no return. Most of these patients would have reported to us had there been a recurrence. Case 4 may be regarded as cured of the attacks. If we look upon the bacteriophage simply as a means of sterilization, it has accomplished its mission when the urine becomes bacteria free, particularly if it remains free for any considerable number of days.

CASE 19—Miss J. S., aged forty-two. C. H. December 31, 1924. Three days ago the patient complained of burning knifelike pain on micturition. This continued unabated. She observed blood in the urine on the morning of December 31. There was no fever or chills. There was a history of two previous attacks, the first several years before, and the second two years before. Urotropin treatment cleared up these attacks. They were preceded by an upper

TABLE II
ACUTE RECURRING INFECTIONS

NO	AGE	SEX	APPROXIMATE DURATION BEFORE TREATMENT WITH BACTERIOPHAGE	URINE BECAME STERILE AFTER BEGINNING TREATMENT IN	NUMBER OF TREATMENTS	URINE MAINTAINED STERILE FOR AT LEAST	RETURN OF BACILLURIA AFTER	METHOD OF ADMINISTRATION	DATE OF EXPLANATION
19	42	F	3 days	3 days	3	4 days	No return	Subcutaneous	12 31 24
20	63	F	4+ weeks	3 days	3	26 days	No return	Subcutaneous	2 19 25
21	9	F		2 days	2	14 days	No return	Subcut B	3 17 30
22	62	F	1 day	9 days	3		7 months	Subcutaneous	12 5 25
22	62	F	1 day	2 days	2	11 days	No return	Subcutaneous	6 19 25

respiratory infection. No known infection preceded the last attack. The urine on December 31 showed — cells, some blood and B coli. Alkalinization was begun. The patient responded promptly to treatment. The urine became sterile in three days. There has been no recurrence to date (1932).

CASE 19

DATE	CELLS	B. COLI	COLONIES	URINE	REMARKS
1925		P	S		
1 18	+++	-	-	acid	R = large regular homogeneous white colonies S = small bluish gray translucent colonies
1 20		-	+	acid	
1 21	+				3 c.c. filtrate in arm
1 22	+	+	+	alk.	3 c.c. filtrate in arm. Symptoms have disappeared
1 23	+	+	-	alk.	3 c.c. filtrate in arm
1 24	-	0	0	alk.	
1 25	0	0	0	alk.	No return of symptoms

CASE 20—Mrs J. W. M., aged sixty three. C. H. On February 19, 1925 the patient complained of inability to hold her urine and burning sensation on urination. These symptoms began four or five weeks previously. She was up frequently at night, and had to void about every two hours during the day. There had been no known recent infections. An ordinary urine specimen showed no pus and no albumin. Santol oil and increased fluids were ordered. The symptoms not improving she was given urotropin for a time and then alkalinization treatment.

On April 30, 1925 the patient reported that while here symptoms might be a little better she still occasionally had attacks of urinary frequency and burning. A catheterized specimen showed pus and B. coli. On May 1 alkalinization was begun. On May 9 the bacteriophage filtrate was satisfactory. Three doses of 3 c.c. each were given on alternate days. The result was marked. The symptoms promptly stopped, and the urine became sterile in three days. On June 7 the urine was negative. It was impossible to make further checks as the patient left for Europe. A careful check was run for us in Geneva on October 11, 1925. The urine sediment was reported negative, and the patient symptom free. She contracted acute dysentery in Egypt February 2, 1927, and died February 9. The laboratory record follows.

CASE 20

DATE	CELLS	L COLI	REMARKS
1925			
5 1	+++	+	Alkalinization begun April 30
5 9	+++		3 c.c. bacteriophage filtrate in arm
5 11	++	+	Arm swelled, painful
5 12		0	3 c.c. bacteriophage filtrate in arm
5 13	++		Retention less marked
5 15	+++		3 c.c. bacteriophage filtrate in arm
5 29	++	0	Cells disintegrating, mostly granular
6 7		0	Symptoms have all disappeared
			Cells still increased

CASE 21—M B, aged nine U II 236520 March 17 1930 Sent to Hospital without a history Patient complained of costovertebral pain and tenderness Had several attacks before Physical examination except for pain on pressure in the left abdomen and reddening of the urethral orifice was negative Temperature 103.8° Respiration 25 Leucocytes 7,500 Duration of the condition could not be determined The urine was loaded with pus and bacilli Urine culture showed +++ B coli

A satisfactory bacteriophage filtrate was developed in eight days Recovery was prompt Sterile urine was found on seven consecutive cultures There has been no recurrence

CASE 21

DATE	CELLS	B COLI	CLINICAL RECORD
1930			
3 18	+++	+++	Alkalinization begun 3 17
3 20	+++		
3 25	+++	+++	
3 27	+++	+++	5 c.c. filtrate subcutaneously, 5 c.c. in bladder
3 28	0	+	
3 29	0	0	
3 31			5 c.c. filtrate subcutaneously, 5 c.c. in bladder
4 1	0	0	
4 2	0	0	
4 3	0	0	
4 5	0	0	
4 6	0	0	
4 10	0	0	Discharged on alkaline treatment

CASE 22—Mrs H M C, aged sixty-two C H On November 11, 1925 the patient was taken ill with pain in the left hip This continued for two weeks, and was diagnosed by her home physician as sciatica She occasionally had aching in this region There were no other conditions that might be related to the complaints at this time

December 5, 1925 On the morning of December 4 she awakened with "general distress" The bladder was distended She passed an unusually large amount of urine This was followed by a stinging sensation in the urethra In 1919 she had had an attack of pyuria with B coli positive, diagnosed pyelitis At this time she had similar symptoms to those

CASE 22

DATE	CELLS	B COLI	REMARKS
1925			
12 5	+++	++	Alkalinization begun
12 11			3 c.c. bacteriophage filtrate in arm
12 12			
12 13			3 c.c. filtrate in arm
12 14			
12 15			3 c.c. filtrate in arm
12 20	0	0	Symptoms disappeared after second injection

previously described A catheterized urine specimen on December 5 showed several hundred leucocytes per low power uncentrifuged field many large clumps Culture showed B coli Alkalinization was begun

The patient continued to be free from symptoms until June 19, 1926 When there was a recurrence of urinary irritation she was again alkalinized and given a second course of bacteriophage injections The urine sterilization was quite prompt as shown in the record This patient has had three distinct attacks The reinfection after previous bacteriophage treatment was promptly overcome by a second series of injections This illustrates that one course of bacteriophage treatment does not build up effective phagocidal antibodies

CASE 22

DATE	B COLI	URINE PH	REMARKS
1926	P S		
6 20	- -	6.5 acid	Alkalinization begun
6 22	- -	7.5 alk	
6 23			3 cc filtrate in arm
6 24	- -	7.6 alk	
6 25	0 0	8.2 alk	2 cc filtrate in arm
6 26	0 0	7.4 alk	
6 29	0 0	7.5 alk	
7 2	0 0	7.4 alk	
7 6	0 0	7.4 alk	

CHRONIC COLON BACILLUS INFECTIONS

In order to form a proper idea of the effect of the bacteriophage in the chronic cases recorded each case should be studied by itself It is possible to group 11 cases together as has been done in Table III in which recovery occurred

TABLE III
CHRONIC COLON BACILLUS INFECTIONS RECOVERY WITHOUT KNOWN RECURRENCE

NO	AGE	SEX	APPROXIMATE DURATION BEFORE TREATMENT WITH BACTERIOPHAGE	URINE BECAME STEPIL E AFTER BEGINNING TREATMENT IN	NUMBER OF TREATMENTS	LAST OPPORTUNITY TO TEST AND FIND URINE STEPIL E	LAST REPORTED RECORDED NO RECURRENCE OF SYMPTOMS	DATE OF ENTRANCE
27	5	F	6 months	2 days	3	4 days	1 month	10 11 26
29	8	F	3 years	1 day	3	6 days	No return	7 8 28
31		F	Many months	6 days	6	12 days	Several months	9 18 28
32	60	F	Many months	513 days	30	320 days	Two years	11 27 28
37	18 m	F	2 months	50 days	6	3 days	No return	7 29 29
39	7	F	4 months	4 days	4	15 days	No return	10 28 29
40	35	F	Many months	5 days	6	4 months	No return	10 29 29
42	2	F	Several months	47 days	3	39 days	No return	8 6 30
45	50	F	Many months	32 days	4	7 days	5 months	5 25 31
37	50	M	30 years	455 days	46	1 month	12 months	6 6 29
34	20	F	Months	1 day*	4	79 days	Two years	1 17 30

*This patient had previously had two courses of bacteriophage treatment without any beneficial effect Four months elapsed between the second and third courses

red without any known recurrence after discharge. The records of two of these, Cases 10 and 12, are of particular interest. One is of over two years' duration the other of probably thirty years. They illustrate very well what persistence in treatment and in cooperation from the patient will accomplish. They seem to preclude the idea of the long result of time, of alkalinization or of both being responsible for the entirely satisfactory result.

There are 6 cases of infections recurring after the first series of treatments. Cases 23, 26, 28, 30, 32, and 44, which finally recovered under the influence of the bacteriophage. These recurrences occurred as early as six days and as late as three and a half months after the first or second series of injections. There are four cases, Cases 25, 38, 43, and 46 which recovered symptomatically, and which, as long as we were able to follow them bacteriologically, continued to show a bacilluria. In all of these there was a modification of the bacilluria or a decrease in the number of bacteria which may be attributable to the bacteriophage. There are three cases (Cases 24, 36 and 41) in which there was no improvement in the bacilluria or in the clinical condition following the administration of bacteriophage. An abridged detailed record of these cases follows.

CASE 23—Mrs J. O., aged fifty-two. C. H. October 6, 1923. The patient complained of a "feeling of pressure" and an occasional sharp piercing pain in the "neck of the bladder." There was no burning or unusual sensation on micturition. These symptoms occurred off and on for a long time. An uncentrifuged urine specimen showed 100 cells per low power field. There had been no previous infections. She had had similar symptoms before. At one time they were associated with fever and what was diagnosed erythema nodosum.

The physical examination was negative as far as its relation to urinary tract infection was concerned. There was a moderate rectocele which was giving no trouble. The meatus urinarius was smooth and not injected. On October 12 a catheterized specimen showed *B. coli*, and an excess of leucocytes. X-ray examination was suggestive of gall bladder disease.

Catheterized urine specimens on October 12, November 19, December 12, 1923, and on January 5, 1924 showed an excess of leucocytes varying from a definite excess with clumps to five or six cells per low power field on January 5. The patient was treated with santal oil for a time, with urotropin and with alkalinization. On March 3, May 22 and June 2 urine samples were free from leucocytes in excess and the patient had been free from symptoms for a long time.

On June 19, 1925 the patient again became conscious of some urinary disturbance. A catheterized specimen showed definite pus and many motile bacilli. Culture showed *B. coli*.

CASE 23

DATE	CELLS	B. COLI	URINE PH	REMARKS
1925				
6 19	+++	+	acid	Resistant type, large convex mucoid colonies
6 23				3 c.c. bacteriophage filtrate in arm
6 24		+	8.0 alk.	Same type of colony
6 25		+	8.0 alk.	2 c.c. bacteriophage filtrate in arm
6 26		+	8.2 alk.	Some flat irregular colonies
6 28				2 c.c. bacteriophage filtrate in arm
6 29		+	7.8 alk.	Colonies all large, very irregular, mostly even
7 3		+	8.8 alk.	Only large flat irregular mostly even type of colonies remain
7 6		+	7.6 alk.	Colonies same as 7.3
7 9		0	7.6 alk.	11 days after last treatment
7 14	0	0	7.8 alk.	16 days after last treatment

Bacteriophage treatment was begun June 23, 1925. Records of the cell content of the urine unfortunately were not kept. Interest was focused on the bacteriology of the urine. The patient's symptoms cleared up and she was much better generally. It will be observed that eleven days after the last injection of the bacteriophage filtrate the urine became sterile and pus had disappeared at least by the sixteenth day. The result of this series of treatments seemed highly successful. There was no recurrence of symptoms for six months.

On January 15, 1926 a recurrence of symptoms after six months occurred. The progress is recorded in the following table.

CASE 23

DATE	CELIS B COLI		REMARKS
1926			
1 15	+	-	Alkalinization begun
1 17	±		3 c.c. bacteriophage filtrate in arm
1 19			3 c.c. bacteriophage filtrate in arm
1 21	++		
1 23	±		Symptoms improved
3 17	+	+	Mild symptoms
3 21	-		3 c.c. bacteriophage filtrate in arm
3 22	+	+	Catheterized specimen
3 23			3 c.c. bacteriophage filtrate in arm
3 24	-		Catheterized specimen
3 25			3 c.c. bacteriophage filtrate in arm
3 26	+		Catheterized specimen
4 2			Catheterized specimen Record lost
5 5	0	±	Symptoms have disappeared
5 13			3 c.c. bacteriophage filtrate in arm
5 14		±	Catheterized specimen
5 15			3 c.c. bacteriophage filtrate in arm
5 17			3 c.c. bacteriophage filtrate in arm
5 18	0		Catheterized specimen Symptoms have disappeared
7 9			3 c.c. bacteriophage filtrate in arm
7 10		±	
7 11			3 c.c. bacteriophage filtrate in arm
7 12	0	±	Catheterized specimen shows a modified atypical organism
7 13			3 c.c. bacteriophage filtrate Symptom free
10 7	++	+	Alkalinization continued Recurrence of symptoms after 3 months
10 12			3 c.c. bacteriophage filtrate in arm
10 14		±	
10 15			3 c.c. bacteriophage filtrate in arm
10 16		±	
10 17			3 c.c. bacteriophage filtrate in arm
10 18		±	
11 5	0	±	Symptoms have disappeared
12 4		0	
1927			
3 22	0	0	Catheterized specimen
3 23	0		Ordinary specimen Symptoms have not returned

Comments—This case illustrates very well the persistence of *B. coli* infections. It also illustrates very well the fact that bacteriophage treatment does not impart immunity, and that a negative culture can be brought about after several recurrences of colon bacilluria. It cannot be said that the urinary tract was ever completely sterilized or whether there had been several reinfections of the urinary tract from the original source. The fact that definite symptoms and definite excess of cells recurred as well as bacilluria, seems to indicate that there was a definite resistant continuous infection present. The presence of colon bacilli was too constant to enable us to attribute their presence to the simple elimination or filtration of organisms which might normally enter the blood stream or the lymphatics and through these channels gain entrance to the urinary passages.

At the time of the first recurrence of symptoms, January, 1926, we had on hand an unusually vigorous polyvalent bacteriophage. We were, however, unable to increase its potency for the patient's strain. A new river water sewage sample was taken. In time we were able to develop a bacteriophage that showed many virers when tested against the patient's strain on solid media in dilutions as high as 1:10,000,000. Both alpha and beta plaques were present.

It will be observed that a prolonged effort over a period of ten months was unsuccessful in causing the organism to disappear from the urine. Its numbers were at times reduced and the patient's symptoms disappeared. After this prolonged effort one would be glad to accept the last two negative cultures (December 4, 1926 and March 22, 1927) as evidence of the efficiency of the bacteriophage treatment. This may be questioned. However, the patient remained free from symptoms for a long time.

The events in this case may lend support to the opinion that the first course of treatment which apparently resulted successfully rendered further use of the bacteriophage useless because of the development of antibacteriophage antibodies. If the body had developed antibodies against the activity of the bacteriophage, it may be that these antibodies would be found in the patient's blood serum. We attempted to prove whether this was so. We ran a series of dilutions of the bacteriophage with the patient's serum. Even in the highest dilutions where only a trace of bacteriophage was present, the serum proved to be innocuous. In no instance did the bacteriophage show any loss of potency over that diluted in a similar way with broth or with normal human serum. Bordet and Ciura* mixed rabbit serum and coliphage together and seeded the mixture with *B. coli*. The colon bacillus grew as luxuriantly as it did in plain broth. This is not analogous to the inoculation of a case of *B. coli* infection with bacteriophage.

An attempt was made on several occasions to demonstrate bacteriophage in the patient's urine. Only at one time, following the first injection (1926) were we able to do so. In this instance large numbers of plaques were found. Their potency we thought was even higher than it was before injection.

CASE 24—Katherine H., aged three. U. H. 242609. November 5, 1925. This was a marked case of nephrosis with a colon infection of the urinary tract of long standing which had resisted alkali and forced fluid treatment. The catheterized urine specimens on November 5, 8, 12, 13, 15, and 17 contained pus and large numbers of *B. coli*. After three subcutaneous injections of 3 cc of bacteriophage filtrate which imperfectly lysed the patient's organism no beneficial effect was secured. Later on a second series of three injections was given. The urine culture became negative and remained so for sixteen days, after which *B. coli* again appeared. We were never able to clear up this infection. Subsequently the

CASE 25

DATE	CELLS	B. COLI	REMARKS
1926			
1 26	+++		Santol oil 5 min t i d Forced fluids
2 5	0		Catheterized specimen Negative No symptoms
4 5			Sudden uncontrollable desire to urinate
1927			
2 21	0	+	
3 2	0	+	Alkalinization begun
3 15			3 cc bacteriophage filtrate in arm
3 17	0	+	3 cc bacteriophage filtrate in arm
3 19	0	+	3 cc bacteriophage filtrate in arm
3 24	0	±	
3 31	0		Symptoms have disappeared
4 14	0		
4 16	0	+	
4 18	0	+	
4 20	0	+	
5 2	0	+	
5 25	0	+	

*Cited by D. Herelle

patient died At autopsy there was no microscopic evidence of involvement of the kidney pelvis

CASE 25—Mrs S, aged thirty C II January 27, 1926 The patient came in with a history of painful micturition and frequency of two weeks' duration, preceded by an acute upper respiratory infection Physical examination was negative aside from the discovery of a small smooth firm pelvic mass the size of a large walnut, separated freely from the uterus and ovary There was a history of a dermoid cyst having been removed two years previously A catheterized urine specimen showed 100 to 120 cells per low power field, no casts, no blood, chemistry normal, B coli —, R type, indicating an old infection

Final Report The symptoms quite promptly disappeared They reappeared October, 1929 We were unable to sterilize this patient's genitourinary tract with three injections of bacteriophage Cystoscopic examination and pyelograms showed no malformations The organism encountered was the R type of B coli At no time did we discover a bacteriophage in this patient's urine

CASE 26—Mrs G W, aged forty eight C II July 18, 1926 The patient had been having nausea, vomiting, fever, and chilliness for a week or ten days, and showed slight but definite jaundice There was no characteristic tenderness in the gall bladder region The symptoms were suggestive of acute gall bladder disease There were no urinary symptoms The patient was very ill By July 28 the patient was much better but still vomited every day, particularly after the noon meal Putting her on thick, smooth food at this time improved this symptom On August 11 the husband wrote that she had made rapid gains The nausea and vomiting had ceased entirely She had been sitting up a little each day, but had developed a burning sensation on micturition On August 14 an attack of vomiting and fever of 103.6° occurred This cleared up in two days

September 7, 1926 The patient entered the Hospital She was too ill to undergo a complete examination She had lost a great deal of weight, was weak and emotional, cried on the slightest provocation The general bed examination showed nothing else No tumor mass could be felt in the abdomen There was no tenderness in the gall bladder region and pelvic examination was negative A urine sample showed no increased cells, the chemistry was negative Later in the day a catheterized specimen was taken for culture It contained pus This was of interest in view of the fact that pus had not been found before Careful check showed that the first sample had not been confused with that of another patient She was put on

CASE 26

DATE	CELLS	B COLI	REMARKS
1926			
9 7	—	+	Santol oil 5 minims after meals
9 9			Bladder irrigated with boric acid sol, 20 per cent argyrol instillation
9 13			Alkalinization begun
9 17			Bladder irrigated with boric acid, 20 per cent argyrol instillation
9 19			Bacteriophage only partially lytic for case strain B coli
9 20			Bladder irrigated and argyrol instillation
9 27			Bladder irrigated and argyrol instillation
9 28			Bladder irrigated and argyrol instillation
10 4	—	++	Patient continues to remain in bed
10 16	—		3 c c bacteriophage filtrate in arm
10 17	—		3 c c bacteriophage filtrate in arm
10 18	—		3 c c bacteriophage filtrate in arm
10 26	+	-	B coli colonies greatly reduced in number
11 1	—	+	Patient up and about Walks down town easily Gaining in strength
11 16	+	+	Has gained 14 pounds in weight Returned home on hexylresorcinol treatment
1927			
1 12	0	-	
2 22		0	
3 10		0	Four months after last bacteriophage injection Patient is very well and active Has gained 18 pounds in weight No recurrence of symptoms

Santol oil pending the development of bacteriophage. We were never able to build a bacteriophage that would completely lyse this patient's organism in the test tube. There was a decrease in the number of organisms following the bacteriophage injections, and the patient made very rapid gains after this time, which we think may be attributed to the bacteriophage. It should be noted, however, that the urine did not become sterile until four months after beginning treatment. This may be attributed to hemolysoreinal. There was a marked effect on the bacterial count ten days after beginning bacteriophage treatment.

Follow Up Record—May 28, 1928. Patient returns on request for further observations. She continues to be well and has no urinary complaints. A catheterized urine specimen, however, showed pus and B coli of two types, large and tiny colonies. One of our stock bacteriophage filtrates was potent for the patient's organism. Alkalinization was begun. Because of this long continued infection we were interested to know if a bacteriophage was living symbiotically with this patient's B coli. We were unable to demonstrate one before beginning the filtrate injections. The following observations were made:

CASE 26

DATE	CELLS	B COLI LARGE	COLONIES TINY	REMARKS
1928		S	R	
5 28	+	+++	+++	Bacteriophage adaption begun
6 22	+	+++	+++	3 cc filtrate in arm. No bacteriophage found in urine.
	+	+++	+++	One hour after treatment. No bacteriophage found in urine.
6 24		0	+++	3 cc filtrate in arm. No bacteriophage found in urine.
		0	++	One hour after treatment. No bacteriophage found in urine.
6 26		0	++	3 cc filtrate in arm.
	0	0	++	One hour after treatment. No bacteriophage found in urine.
6 29		0	+	3 cc filtrate in arm. No phage in urine after 24 hours. Abundant after 48 hours.
		0	+	One hour after last treatment. No phage in this sample of urine after 24 hours. Abundant plaques after 30 hours.

These observations are of interest in that the sensitive strain or type of B coli in this patient's urinary tract was easily caused to disappear. The tiny or resistant type persisted although its numbers were definitely reduced. They also show that the bacteriophage after being injected subcutaneously may later appear in the urine. In this case it required thirty to forty eight hours before it could be demonstrated. The bacteriophage filtrate used in this patient was potent for several case strains of B coli in dilutions up to one to ten thousand.

CASE 27—Dorothy D, aged five. U H 155247. October 11, 1926. The patient was admitted to the Hospital because of albuminuria, casts, and pus in the urine. In April, 1925 she developed a fever without any other symptoms, which continued for two months. There was considerable loss of weight. The fourth week of this illness casts and albumin in the urine were discovered. These findings persisted. There was no urinary frequency, no hematuria, no edema, nocturia or incontinence. Recurring attacks of dull pain in the left upper abdomen have occurred since June, 1925. These sometimes last for twenty four hours. No vomiting or fever. Last attack occurred two weeks previous to entrance.

Physical examination. Evidence of loss of weight, otherwise negative. Blood hemoglobin 75 per cent, red cells 3,400,000, white cells 8,100. X-ray of chest negative. Upper respiratory infection present. "Cold."

November 23, 1926. Mother reported a good weight increase, better color, exceptional appetite. Prior to treatment appetite was very poor.

CASE 27

DATE	CELLS	B COLI	REMARKS
1926			
10 11	++	+++	
10 22			Alkalinization begun
10 23	++		3 c c filtrate in arm Marked reaction Temp 104° swelling, redness, pain in leg
10 25	±	0	2 c c filtrate in arm Reaction less marked
10 27	0	0	3 c c filtrate in arm Urine alkaline
10 29	0	0	

CASE 28—Mrs W H A, aged fifty C H October 17, 1927 Several days ago the patient began to complain of painful micturition A catheterized urine specimen showed many leucocytes, no crsts, and on culture — B coli Otherwise this patient is in excellent health Alkalinization was begun It is possible that she previously had a similar infection

CASE 28

DATE	CELLS	B COLI	REMARKS
1927			
10 17	+++	+++ S	Sensitive or lytic type of colony Alkalinization
10 18	+++		
10 19	+++		
10 20	+++		
10 21			3 c c filtrate in arm (diluted filtrate)
10 22		0	
10 23	+	0	3 c c filtrate in arm
10 25	+++		3 c c filtrate in arm
10 27	+++	0	
11 1	+++	- R	Resistant type of colony has developed
11 3	+++	±	3 c c filtrate in arm (undiluted filtrate)
11 5	++	±	3 c c filtrate in arm (produced marked local reaction)
11 7			3 c c filtrate in arm
11 9	+++		
11 14	++		Argyrol instillation into bladder (25%)
11 29	0	0	Symptoms have disappeared

The change in the character of this patient's urine after the first dose of bacteriophage was remarkable The symptoms were also improved There was a greater improvement in the bacteriology than in the cytology The urine remained sterile for nine or ten days when it again became positive for B coli An entirely different organism than the original one was now present, a typical rough bordered colony, the so called R type, the organisms of which were self agglutinating On November 29, thirty nine days after the first dose of bacteriophage, thirty four days after the last, a catheterized urine specimen was negative bacteriologically and cytologically The patient continued to be well However, the clearing up of the urine was preceded fifteen days by an instillation of argyrol, at which time a culture test was not run In February, 1928, after a symptomless period of four months, there was a recurrence of the former symptoms

CASE 28

DATE	CELLS	B COLI	REMARKS
1928			
2 28	—	-	Alkalinization begun
3 11			3 c c bacteriophage filtrate in arm
3 13			3 c c bacteriophage filtrate in arm
3 14	0	0	
3 16			3 c c bacteriophage filtrate in arm
3 17	0	0	Arm sore, swollen, chilliness aching

The response to bacteriophage again was rapid. It is instructive to follow the course of this case still further. On September 17, 1928 the patient complained of urethral irritation. Examination revealed a slightly irritated urethral caruncle, which responded to cauterization with silver nitrate. In November irritation again developed with excess of leucocytes and red blood cells in the urine. A catheterized specimen was negative for cells. They were coming from the meatus. This situation improved on a second cauterization. On November 21 irritation and increased cells occurred again. A catheterized specimen was negative for cells. The caruncle was removed by electrocoagulation. On December 7, 15, January 9 and 29, 1929, urine samples remained cytologically negative. On April 30, and May 27 specimens were negative. On June 3 and 22 ++ cells were present, July 29 negative, December 3 negative, January 7, 1930 cells +++, B coli negative, January 11 B coli negative. On April 25, 1931 small polypoid growths in the urethra, slightly irritated, were found, but none of the symptoms associated with the former bacilluria were present.

This case is cited at length to show that it is wise to differentiate the causes of urinary symptoms and findings in cases that have been treated with bacteriophage. One might easily in this case have made up his mind that after all the bacteriophage accomplished little—if he trusted the ordinary urinary findings and the symptom of urethral irritation. Inspection and catheterization are necessary.

CASE 29—Irene R, aged eight U H 197384 July 8, 1928 Patient brought to Hospital for tonsil and adenoid removal. Three years ago appetite became poor. On several occasions she vomited after heavy meals. Riding on street cars nauseated her. Aside from septic tonsils and evidence of mental retardation the examination was negative. Kahn negative. Bed wetting began about three years ago.

September 12, 1928 Night sweats for past two weeks, at which time she began to have chills, followed by lassitude and loss of weight. Nausea followed the chills. Complained of pain in right chest when she breathed, afternoon fever, and drenching sweats. Examination revealed tenderness in the right lower quadrant of the abdomen with muscle spasm, slight general adenopathy, decreased breath sounds, right axillary line near nipple level. Tuberculin negative. X ray of lungs showed an increase in the hilum shadows not typical of tuberculosis. Blood pressure 110/90.

Blood chemistry chlorides 474, serum protein 7.6, albumin 4.8, globulin 2.8, cholesterol 0.208, phenolsulphonephthalein negative, nonprotein nitrogen normal.

October 7, 1928 Urine showed cells +++, B coli ++++. Three stains for tubercle bacilli negative. Pig inoculated (result was negative). B coli positive. Condensed record follows.

CASE 29

DATE	CELLS	B COLI	CLINICAL NOTES
1928			
10 7	++++	++++	Alkalinization begun
10 8	++++	++++	Phage development begun
10 10			Temp 102° Restless, eyes puffy
10 11	++++		
10 13	++++	++++	
10 20	++++	++++	
10 24	++++	++++	
10 31	++++	++++	
11 8	++++	++++	
11 10			Urotropin and sodium acid phos Alkali begun again Nov 17, 1928
12 11	++++	++++	3 c.c filtrate in arm, 3 c.c in bladder Very active phage
12 12	0	+	20 hours after treatment
12 13			3 c.c filtrate in arm, 5 c.c in bladder
12 14	0	0	No phage could be recovered from urine
12 15			3 c.c filtrate in arm, 5 c.c in bladder
12 16	0	0	
12 17	0	0	
12 18	0	0	
12 21	0	0	

Bacteriology—Three times this patient went through the ordinary process of alkalinization preparatory to bacteriophage treatment and for some unexplainable reason the filtrate was not given. This alkalinization had no effect in lessening the number of *B. coli* or in changing their colony characteristics. Several methods of treatment were tried without benefit. The bacteriophage filtrate was potent six weeks before it was used for treatment. During this time its titer of potency was constantly raised by successive feedings. It became highly polyvalent and was used as a base for other cases. Its action after subcutaneous injection and instillation was very energetic. It caused the pus to disappear from the urine in twenty hours after the first injection and the number of organisms to change from 10^{11} to 10^0 . Twenty-four hours after the second injection the urine was sterile and remained so.

CASE 30—Andrew W., aged eleven. U H 198774 July 24, 1928. Patient came to the Hospital because of a sore mouth. On July 21 the patient had a chill, and on the following day had chills, fever, delirium, and malaise. These symptoms continued for three days. He complained of his teeth, mouth, and tongue being sore. The mother described sores on the tongue and buccal mucous membranes. He also told that one week previous a cousin with whom the patient had been playing had the same mouth infection.

On entrance small white patches were found on the upper left tonsil, an ulcerated spot on the buccal mucous membrane, and several small ulcerated patches on the gum margins. There was a marked left cervical adenitis. Aside from this and the general appearance of illness the examination was negative. X ray of the chest was negative. Temperature 102° .

August 9, 1928. Smears from the ulcerated surface were negative. Repeated urine examinations were negative. Patient discharged. Diagnosis: ulcerative stomatitis.

August 29, 1928. Readmitted. There had been an attack of fever, nausea and delirium in November lasting four days. Two similar attacks had occurred since that time. Patient was fairly well between these attacks. During the last six weeks there had been puffiness of the eyes. There had been urinary frequency and burning at times. On August 28, 1928 there was right costovertebral angle pain, fever and delirium. Patient was poorly.

CASE 30

DATE	CELLS	B. COLI	URINE FINDINGS—CLINICAL NOTES
1928			
8 30	+++	—	
9 16	—	++	Bacteriophage development begun
9 20	—	—	
9 23	++	—	
9 24	—	+++	
9 26	+++	+++	
10 8	+++	+++	Control for treatment not satisfactory. Moderately potent phage developed.
10 9	+++	+	3 c.c. filtrate subcu. Severe general and local reaction. Temp 103.5°
10 10	—	+++	3 c.c. filtrate subcu. Severe general and local reaction.
10 11	—	+++	
10 12	—	—	3 c.c. filtrate subcu. Severe general and local reaction.
10 13	+	—	
10 14	0	+	
10 16	0	—	
10 21	—	—	
10 26	+	+	3 c.c. filtrate subcu. Severe general and local reaction.
10 28	—	++	
10 30	0	—	
11 3	+++	+++	
11 4	—	—	
11 5	—	—	
11 6	+++	+++	
11 7	—	—	
11 12			Cystoscopic. Left pyelogram. No pelvis retention. Right pyelogram. Slight dilatation of ureter. Patient has had alkalinization treatment all this time.

CASE 30 (Cont'd)

DATE	CELLS	B COLI	URINE FINDINGS—CLINICAL NOTES
1929			
8 29			Discharged on sodium bicarbonate for fourteen days with four day intervals without carbonate
1930			
3 10			Readmitted Repeated febrile attacks since discharge
3 12			Tonsillectomy and adenoidectomy
3 15			Discharged from Otology Service
3 30			Readmitted High fever past five days Urine pus No casts No alb
4 10	+	+++	New phage Very potent for present clinical strain Filtrate has been under constant development since last treatment Alkalies have been given continuously
4 29	++	+++	3 cc filtrate subcutaneously, 5 cc in bladder
4 30	+	±	Marked local and general reaction
5 1	±	±	Missed treatment
5 5	++	+++	3 cc filtrate in arm, 5 cc in bladder Reaction marked
5 6	++	+++	
5 7	±	±	0 cc filtrate in arm, 5 cc in bladder Reaction none
5 8	0	0	3 cc filtrate in arm, 5 cc in bladder Reaction marked
5 9	0	0	
5 10	0	0	
5 11	0	0	
5 12	0	0	
5 16	0	0	
5 19	0	0	
5 21	0	0	
5 22			3 cc filtrate in arm, 5 cc in bladder Reaction marked
5 23	0	0	
5 24	0	0	
5 25			Discharged on alkaline treatment
7 29	0		Returns for check Urine not sent for culture Returned home
1931			
2 11	0		Returns Several attacks of gradually increasing nervous manifestations Very irritable Pain in right leg and costovertebral angle for 2-3 weeks Urine not sent for culture

nourished, looked chronically ill There was puffiness of the eyes and tenderness in the right flank Tonsils were septic Kahn negative

August 30, 1928 Urine showed pus and B coli +++

This case illustrates the necessity of securing an unquestionably potent bacteriophage for treatment From August 30, 1928 to April 29, 1930, a period of twenty-two months, no change in the number or virulence of B coli was observed after twenty cultures, excepting following the first series of bacteriophage treatments with a filtrate we recognized as unsatisfactory Two days after the third injection the culture changed from +++ to ± and the cells from +++ to 0 The original picture however, was again established in two weeks

CASE 31

DATE	CELLS	B COLI	REMARKS
1929			
9 18	++	+++	3 cc filtrate in arm
9 19	+++	+++	
9 20	+++	+++	3 cc filtrate in arm
9 22	++	++	3 cc filtrate in arm
9 24	0	0	3 cc filtrate in arm*
9 26	0	0	3 cc filtrate in arm
9 28	0	0	3 cc filtrate in arm
10 6	0		Discharged

*Through a misunderstanding patient stopped soda when bacteriophage treatments were begun Resumed

The entirely satisfactory bacteriophage filtrate given April 29 and May 3, 1930, brought about prompt sterilization of the urine. The urine remained sterile on ten successive cultures over a period of sixteen days after which the patient was discharged.

CASE 31—Mrs B aged 22 C H September 18, 1928 Sent to Hospital for bacteriophage treatment for pyuria and bacilluria which has resisted other forms of treatment for many months. She has been taking soda for several days preparatory to treatment. The urine is alkaline. There is a slight excess of cells. She is now feeling better because of the soda. A satisfactory phage was found among our stock filtrates. Recovery was prompt without recurrence.

CASE 32—Mrs J D aged sixty C H November 30, 1928 Patient has been under treatment for pyuria for many months. It is not possible to determine the exact duration of the infection. Urotropin hexylresorcinol and infusion of bucu treatments have been employed. The pyuria has partially cleared up but the bacilluria continues. She is conscious at times of urinary irritation and occasionally of urinary frequency. There has been a bad odor to the urine for a long time.

This patient came under our observation because of bronchial asthma which was found to be on a multiple sensitization basis. Only the record of her urinary infection is recorded. Aside from the slight but definite incapacity of age incident on these two conditions the physical examination has no bearing on the urinary infection. The urine contained pus and many bacteria. The first catheterized specimen was taken November 26, 1928. It showed +++ cells and +++ B coli. On Endos media the colonies were 2 mm in diameter and were characteristic of B coli. The organism reacted to dextrose, lactose, saccharose and on dulcitate for B coli communior characteristically.

Bacteriophage adaptation was very difficult in this case. On constant planting from November 27, 1928 until July, 1929 only a bacteriophage of weak potency was developed. This was given with the hope that it might be enhanced in the patient*. A perusal of the treatment record will show that nothing was accomplished in a period of ten months excepting in the cell content of the urine and statements from the patient that she was better. In fact, she was more encouraged than we were.

In September, 1929 we obtained the first bacteriophage that seemed satisfactory. It was very potent for the patient's organism. There was a prompt clearing of the patient's urine. The catheterized specimen showed no excess of cells two days after the first subcutaneous injection. After the third injection bacteria in the direct drop were negative, and the culture dropped from +++ to +. From this time on the cell content was normal but the organisms were slow in disappearing. The urine finally became sterile and the patient has remained symptom free until the last report on January 4, 1932.

The patient was treated for over one year before any remarkable change occurred in the bacilluria. Consequent on the securing of a potent bacteriophage marked improvement began. Alkalinization was kept up almost constantly. The detailed treatment record follows.

CASE 32

DATE	L P * CULTURE			FILTRATE		REMARKS
	CELLS	BACT	B COLI	S Q	BLAD	
1928						
11 27	+++	+++	+++			Marked urine odor Alkalinization begun Stock phage No effect on patient's B coli Special filtrate building
12 5	+++	+++	+++	3 cc		
12 7	+	+++	+++	3 cc		
12 9	+	+++	+++	3 cc		
12 12	+	+++	+	3 cc		
1929						
1 3			+++			
1 8				3 cc		
1 12	++			3 cc		

Low power

*Cowie D M Observations on the Bacteriophage Ann Clin Med 1 73 1926

CASE 32 (Cont'd)

DATE	L P * CULTURE			FILTRATE		REMARKS
	CELLS	BACT	B COLI	S Q	BLAD	
1929						
3 30	+	+++				
4 10	++++		++++			
4 28	++++		++++			
5 6	+	+++				
5 22	++++	++++	++++			More cells and bacteria than seen before
6 12	++++	++++	++++			
6 20			++++	3 cc		First injection second series Bacteriophage still weak
6 22	++	0	++++	3 cc		
6 24	±		++++	3 cc		
7 10	++		++++	3 cc		Weak phage used third series
7 12	+++		++++	3 cc		
7 14	0	0	++	3 cc		
7 15	0	0	0			
7 24	++	+++	++++	3 cc		Weak phage fourth series
7 26	+++	+++	++++	3 cc		
7 29	+	+++	++++	3 cc		
9 5	++	+++	++++	3 cc		Fifth series filtrate very potent First obtained potent for patient's organism Constant development since Nov 27, 1928
9 7	0	++	++++	3 cc		Urine odor has disappeared
9 9	0		++++	3 cc		
9 10	0	0	+			24 hours after third injection
9 11			±	3 cc		
10 8	0		++++	3 cc		6th series Filtrate being enhanced by feeding every day
10 10	0		+++	3 cc		
10 12			+	3 cc		
10 14	0		+++	3 cc		
10 19	0		+++	3 cc		
10 25	+		+++	3 cc		
11 1	0		++++	3 cc		
11 8	0		*+++	3 cc		
11 15	0		+++	3 cc		
11 27	0		++++	3 cc		
12 6	0	0	+			
12 20	0	0		3 cc		
1930						
1 3	0	0	++	3 cc		
1 14	0	0	0	3 cc	10 cc	
1 21	0	0	0	3 cc	10 cc	
1 28	0		±	3 cc	10 cc	Two colonies B coli
2 5	0		0			
4 23	0	0	+			
4 30	0	0	0	3 cc	10 cc	
5 7	0	0	±			An entirely different organism
5 14	0	0	0	3 cc	10 cc	
6 4	0	0	0			
12 4	0	0	0			No further symptoms Recent urine sample shows no excess leucocytes

*Low power

CASE 33—Mary J N, aged seven C H April 26, 1929 The patient has complained of bed wetting and urinary frequency spells lasting for two weeks at a time with remissions for variable periods This situation has been going on for many months Pus in the urine has been reported There has been no fever The patient is extremely nervous and excitable during the bed wetting periods and has frequent nose bleeds The remissions may be as long as six months, during which time her irritability disappears The attacks begin with suddenly developing uncontrollable urination and urinary frequency If at these times she endeavors to hold her urine it causes great discomfort There seems to be no background for the urinary infection She is a robust youngster and has had none of the

contagious diseases and no upper respiratory infections. A catheterized urine specimen showed a great excess of leucocytes and *B. coli*. Alkalinization begun.

CASE 33

DATE	CELLS	B. COLI	REMARKS
1929			
4 26	—	+++	Alkalinization begun
5 17	0	—	
5 28			3 cc filtrate in arm
5 29	0	—	
5 30			3 cc filtrate in arm
5 31		+++	
6 1			3 cc filtrate in arm
6 2		—	
6 10	—		Catheterized urine specimen
6 13	±		Catheterized urine specimen
6 15		—	
6 21	—	±	

We had great difficulty in treating this child. We had to discontinue the injections because of poor cooperation. The injections we gave were forced. There was noticeable improvement in the bacterial count. She was put on urotropin treatment for two weeks, then back on alkalinization. On September 9, 1929 the mother wrote that the child had had one of the severest attacks she had ever witnessed. Advised return once a week for bacteriophage treatment. The record follows.

CASE 33

DATE	CELLS	B. COLI	REMARKS
1929			
9 16	—		Alkalinization continued
9 28	—		
10 7	±	—	
10 12	0	—	
1930			
1 3			3 cc filtrate in arm 10 cc in bladder
1 4		0	
1 11	—		3 cc filtrate in arm 10 cc in bladder
1 18	±		3 cc filtrate in arm, 10 cc in bladder
1 25	0		3 cc filtrate in arm, 10 cc in bladder
2 15	0	++	Gave three doses to be given at home
4 4	0	0	

It will be seen that the bacterial count has continued to be decreased. The day following the first combined treatment the urine became sterile. Because of the difficulty in securing the child's cooperation catheterization had to be abandoned. On February 15, 1930 we succeeded in securing a specimen. It showed — *B. coli*. Three doses of bacteriophage were given to be administered at home. On April 4, 1930 the urine was sterile and the patient had been free from symptoms since January. Even though there was little improvement noted following the first series of subcutaneous injections the parents, an usually intelligent people, say she was always improved in a general way. This was more marked following the second series. She was very much less irritable.

CASE 34—Caroline P., aged twenty. C. H. The patient has been under observation since 1914, when she was five years old. At this time she gave a history of febrile attacks of seven to ten days' duration, coming on periodically since early infancy. The fever ranged between 101° and 102°. The attacks stopped spontaneously and recurred at intervals of three to seven months. One attack was accompanied by severe chills and cyanosis. Examination revealed an enlarged left kidney, pyuria with large numbers of mononuclear

cells (lymphocytes) In the absence of tubercle bacilli this finding led to the diagnosis of tuberculosis of the kidney, which was proved a year or two later by finding tubercle bacilli and by nephrectomy She was studied again in 1925, albuminuria was found There was no increase in cells Guinea pig inoculation was negative for tuberculosis In 1926 and 1927 albuminuria was + and ++ with only slight excess of cells In 1929 albuminuria was still present and many B coli There were no symptoms referable to the urinary tract An entirely satisfactory bacteriophage was not obtained after a long effort We decided to try it and continued to enhance its virulence The urine finally became sterile two days after we secured an entirely satisfactory filtrate The patient had a very severe reaction following the injection on January 22

CASE 34

DATE	CELLS	B COLI	REMARKS
1929			
6 5	++	+++	Alkalinization begun First series
7 23			3 cc bacteriophage subcutaneously
7 25	0	+++	3 cc bacteriophage subcutaneously
7 27			3 cc bacteriophage subcutaneously
7 29		+++	No improvement in bacilluria
9 17	0		3 cc bacteriophage subcutaneously Second series
9 19			3 cc bacteriophage subcutaneously
9 21			3 cc bacteriophage subcutaneously
9 23	0	+++	No improvement in bacilluria
1930			
1 17	0	+++	
1 18	0		3 cc bacteriophage subcutaneously, 10 cc in bladder Third series
1 20	0	0	3 cc bacteriophage subcutaneously, 10 cc in bladder
1 22	0	0	3 cc bacteriophage subcutaneously, 10 cc in bladder
4 9	0	0	3 cc bacteriophage subcutaneously, 10 cc in bladder

CASE 35—A B C, aged fifty C H June 6, 1929 Patient came in with a history of pyuria dating from an attack of typhoid fever in 1895 (thirty four years ago) which was complicated by a severe cystitis Pus has not completely disappeared from the urine since this time In 1896 kidney stones were removed The operation was not entirely successful and a second operation was done in 1898 The pyuria has been more marked since this time If the patient is careful to drink large quantities of water he gets along very well, but the bad odor continues and is noticeable at all times in his bathroom He has no other complaints He looks well and feels well He is anxious to have the urinary infection cleared up, particularly the bad odor

Since his operations he has been up at night two or three times to urinate About once a year he has a digestive upset which is characterized by nausea, vomiting, and high fever This sends him to bed for a week There are no urinary symptoms at these times However, if he does not persist in drinking comparatively large amounts of water there will be irritation in the bladder and bad odor to the urine Colon bacilli were found in the urine by a competent observer in 1899 The last time they were looked for, eight or ten years ago, they were still present

Past Treatment In addition to a large water intake, urotropin has been taken more or less systematically for many years It finally had to be discontinued because it "irritated the stomach"

Physical Examination Patient was well nourished and well muscled The tonsils were in, no history of infection Teeth were negative Joints were negative The lungs and heart were negative Blood pressure 118/90 Abdomen negative Appendix scar—1927 Genitals and prostate negative Slight pruritis ani

Urine Turbid yellow, acid, 1 020, odor of infection No albumin No sugar The sediment showed many motile bacilli Several samples gave the same findings The gravity varied from 1 009 to 1 019 on the Mosenthal test The night output was slightly greater than the day

Bacteriology A catheterized specimen showed four distinct strains or types of *B. coli*. Colonies developed on Endo- plates as follows:

No. 1 Large luxuriant flat colonies 2 mm in diameter with red metallic scum, visible in twenty four hours.

No. 2 Medium sized colonies 1 mm in diameter with red metallic scum in twenty four hours.

No. 3 Small colonies with mucous surface. No metallic scum in twenty four hours. Mucous surface and red metallic scum in forty eight hours.

No. 4 Tiny colonies showing faint scum with hand lens in seventy two hours.

Tiny colonies showing white in nine six hours.

Tiny colonies showing pink in one hundred twenty hours.

Tiny colonies showing red metallic luster in one hundred forty four hours.

When subcultured on Endo- medium these colony types always develop in the same manner. Transplants of Type 1 remained the same for over seven days. After four months transplants behaved in the same manner. Types 2 and 3 showed no change with the same technic. Type 4 after alternately transplanting in broth and on hard media showed no change in its colony characteristics. One specimen on continuous incubation without opening the Petri dish for five days showed two typical large Type 1 colonies. The following morning the sixth day two more Type 1 colonies had developed. These large colonies possessed all the characteristics of Type 1. When transplanted, they developed in twenty four hours and showed a red metallic scum. We have never been able to produce small or tiny colonies from Type 1 colony. These colony types always run true to form.

On three occasions we have transplanted tiny, Type 4 colonies into broth for twenty four hours, then back onto Endo- plates, resulting in nearly a pure culture of Type 1 which covered the plates. On one occasion it was five days before large colonies appeared, on two occasions it was three days before large colonies appeared on Endo-.

On blood agar all colony types become distinct white colonies in twenty four hours. Type 4 is barely visible to the naked eye (0.5 mm). It attains its normal size, 1 mm, in diameter, only after forty eight hours.

In broth a faint haze is produced with Types 1, 2 and 3 in twenty four hours. It is difficult to carry Type 4 through a broth transplant without change in form even though the transplants are made every nine hours. Thus it will be seen that this type which is the most difficult to irradiate from the patient's urinary tract readily develops into Type 1 under proper cultural conditions.

Action on Sugars Types 1, 2, 3 and 4 ferment dextrose and lactose. They do not ferment saccharose.

Developing the Bacteriophage—Sewage base was inoculated with the patient's organisms on June 6, 1929. Feedings and filtrations were done every 12 hours for 35 days before any favorable lysis was produced, and then only on Types 1, 2 and 3 (July 11, 1929). Feedings and filtrations were continued until September 4 before a phage was obtained that was active for Type 4. As will be seen from the treatment chart it was not difficult to develop a bacteriophage for the first three types with the exception of our first effort. It has been more difficult to secure a filtrate lytic for Type 4. None of our stock filtrates were effective.

Treatment of case with phage filtrate—As previously pointed out, bringing the P_H of the patient's urine to the neutral or slightly alkaline point is an essential feature in the treatment of colon infections of the urinary tract with Coli phage. We were fortunate in having a patient who could give us perfect cooperation. It has been necessary to omit treatments for long periods of time on several occasions. This has given us opportunity to investigate the permanency of bacteriophage sterilization.

Subcutaneous Injection—As a matter of interest a dose of filtrate was given subcutaneously June 14. It had not been developed to a satisfactory point at this time. Cowie has observed that occasionally filtrates that are not completely lytic in vitro may affect the organism when given subcutaneously. There was no such action in this instance. However twenty four hours after the second dose Types 1, 2 and 3 disappeared from the urine.

and have continued to be absent, with the exception of one occasion for a year and nine months (April 7, 1930) Type 4 was definitely affected twenty four hours after the third dose of filtrate (July 16), but was as resistant as ever eight days later despite the fact that two additional treatments had been given and the filtrate was being enhanced daily during this time. On September 4 the first bacteriophage active for Type 4 was obtained. The first negative Type 4 culture was obtained December 2, 144 days after the first of fourteen 3 cc subcutaneous injections. The urine remained free from Type 4 for five days, perhaps longer, but showed a ++ growth on the eighth day (December 10), a + growth on the twenty fifth day, a negative growth on the thirty first day. The urine was found completely sterile to all types for fifteen days thereafter. The patient went to Florida, returning thirty five days later. At this time, he was symptom free the urine was fairly clear, but it gave a +++ growth of Type 4 colonies. This type persisted for twenty seven days and became negative after the eighth treatment.

Bladder Instillation—It was our purpose to gain as much information as possible on the effect of the filtrate by subcutaneous injection. Bladder instillations were not added until December 27, 1929. After the bladder was emptied, without bladder irrigation, 10 cc of filtrate were instilled. The patient was asked to hold his urine as long as he could comfortably. At first he remained in bed, but after a few trials he was able to do as well by being about. He could hold the filtrate from four to six hours. There has been greater improvement since this time, but the reader will decide for himself by reference to the treatment chart whether this is due in greater part to the bladder instillation or to persistence in treatment. Our feeling, from work on other cases, is that the double method is the better one.

Cells—The patient's urine at the beginning of observation was definitely cloudy. The uncentrifuged specimen showed clumps and an excess of leucocytes which we marked as ++++. The urine remained in this condition until after the third subcutaneous injection of bacteriophage filtrate. Seldom since this time has there been any appreciable cloudiness. On a few occasions the morning urine, following a bladder instillation the evening before, has been cloudy because of an amorphous detritus. On such occasions the urine was found to be sterile.

Odor—There had been an unpleasant odor to the patient's urine since his kidney operations in 1896 and 1898. As previously stated this was marked enough to produce a characteristic odor to the bathroom. The odor could be lessened by drinking freely of water. By reference to the treatment table it will be seen that the odor became much less after the third subcutaneous injection of bacteriophage filtrate. It had completely disappeared November 26, 1929, one hundred four days after the first treatment (12 treatments in all, 36 cc of filtrate). At this time the patient volunteered the information "That is the first time my urine has been free from odor in 30 years." It was actually thirty three years. The odor has not returned. The urine for the most part has been clear as a normal urine—influences aside from pyuria at times have imparted a cloudiness.

Treatment—The patient has taken soda constantly since beginning treatment. The total period covers about two years. There may have been a few days at a time when soda was omitted. Three level teaspoonsful a day has been the dose. Oranges and grape fruit have been taken freely, and during the past year milk sugar. This regimen has often resulted in a urine alkaline to litmus, more often neutral. There have been no symptoms attributable to the soda. There has never been any edema.

Bacteriophage Filtrate—A great effort has been made to secure filtrates as free as possible from color and protein. Redness, heat, and swelling frequently occurred at the site of injection, more often when the color was definite and when the dilution of the broth was not as great as it should be. The best filtrates were those developed with synthetic media, and those that could be developed to a high phage titer and which could consequently be more highly diluted. By reference to the treatment table it will be seen that in the first period of observation, June 6, 1929 to January 18 1930, two hundred twenty five days or seven and one half months, we were successful in promptly overcoming B coli Types 1, 2 and 3. Three doses of filtrate of 3 cc each accomplished this. Type 4 disappeared De

cember 2 eighty six days after the first of 5 treatments with a potent bacteriophage. The urine remained practically sterile to all types for forty seven days when this period of observation ended. There was now an interval of one month. The second period of observation continued for over six months from February 22 to September 4, 1930. At the beginning of this period the first three types were continuously absent from the catheterized specimen. There was however a \pm growth of Type 4. After 5 subcutaneous injections and bladder instillations the urine became sterile but from time to time Type 4 appeared as will be seen by consulting the chart. An interval of four months elapsed before another observation was made. The urine was sterile to all types. There followed an interval of two months without treatment at the end of which Type 4 was \pm . It responded well to treatment but it frequently reappeared in the urine.

The third period of observation covered ninety eight days from May 29, 1930 to September 4, 1930. At the beginning of this period the urine was still clear and odorless. On direct examination a few motile bacilli were observed. A combined treatment with the previous filtrate which had been developed further was given. The patient could not return for ten days. At this time the urine continued to be negative to Types 1, 2, and 3, and showed a \pm culture of Type 4. A week later the urine was again negative for all four types and remained so for at least five days showing only a few organisms on the eleventh day, increasing to \pm on the twenty fifth day in spite of three combined treatments.

It will be seen that during the course of these observations one might have been satisfied that the patient was cured had he been discharged January 18, 1930 or particularly had he been discharged November 7, 1930, when two urine samples were negative to all four types four months after the last treatment.

CASE 35

DATE	CLEAR OF CLOUDY	ODOR	CELLS	DIPECT BACT	CULTURE TYPES				CLINICAL NOTES
					1	2	3	4	
1929									
6 6	Cloudy	Foul	—	—	++	+	—	—	Alkalinization begun
6 12	Cloudy	Foul	—	—	—	+	—	—	Alkalinization begun
6 13	Cloudy	Foul	—	—	—	—	—	—	Alkalinization begun
6 14	Cloudy	Foul	—	—	—	—	—	—	3 cc in arm Stock bacteriophage
6 15	Cloudy	Foul	—	—	++	—	—	—	
7 11	Cloudy	Foul	—	—	++	+	—	—	3 cc in arm Filtrate lytic for 1, 2 and 3
7 13	Cloudy	Foul	—	—	—	—	—	—	3 cc in arm
7 15	Clear	Foul	0	—	0	0	0	—	3 cc in arm
7 16					0	0	0	—	3 cc in arm 18 hr after third phage
7 19	Clear	Sl *							3 cc in arm
7 22	Clear	Sl							3 cc in arm
7 24	Clear	Sl	0		0	0	0	—	3 cc in arm
7 25	Clear	Sl			0	0	0	—	
7 30	Clear	Sl			0	0	0	—	3 cc in arm
8 6	Clear	Sl			0	0	0	—	3 cc in arm
9 4	Clear	Sl	0		0	0	0	—	3 cc in arm First phage found active for 4
9 6	Clear	Sl							3 cc in arm
9 9	Clear	Sl							3 cc in arm
9 11	Clear	Sl	0		0	0	0	—	
9 25	Clear	Sl			0	0	0	±	
10 18	Clear	Sl	—	—					
11 26	Clear	0	±		0	0	0	—	3 cc in arm
11 29	Clear	0							3 cc in arm Urine unusually clear "First time free from odor in 30 years"
12 2	Clear	0	0		0	0	0	0	
12 6					0	0	0	0	
12 7					0	0	0	0	

*Slightly foul

CASE 35 (Cont'd)

DATE	CLEAR OR CLOUDY	ODOR	CELLS	DIPECT BACT	CULTURE TYPES				CLINICAL NOTES
					1	2	3	4	
1929									
12 10	Clear	0	++					++	3 cc in arm
12 27	Clear	0			0	0	0	+	3 cc in arm, 10 cc in bladder
1930									
1 3	Clear	0	0		0	0	0	0	3 cc in arm, 10 cc in bladder
1 4	Clear	0			0	0	0	0	3 cc in arm, 10 cc in bladder
1 6		0			0	0	0	0	
1 10		0			0	0	0	0	3 cc in arm, 10 cc in bladder
1 11	Cloudy	0	0	0	0	0	0	0	Amorphous detritus Held filtrate all night
1 17	Clear	0	0	0					3 cc in arm, 10 cc in bladder
1 18	Clear	0	0	0	0	0	0	0	Went to Florida
			<i>Interval of One Month Since Last Treatment</i>						
2 22	Clear	0	+	+	0	0	0	+++	3 cc in arm, 10 cc in bladder Re turned from Florida
2 24	Clear	0	0	0	0	0	0	++	3 cc in arm, 10 cc in bladder 48 hr after phage
2 28	Clear		++	++	0	0	0	+++	3 cc in arm, 10 cc in bladder Be fore treatment
3 4	Clear	0	0	0	0	0	0	+++	3 cc in arm, 10 cc in bladder Be fore treatment
3 5	Clear	0	0	0	0	0	0	+++	18 hr after last phage
3 7	Cloudy	0	+	0	0	0	0	++	3 cc in arm, 10 cc in bladder Be fore treatment
3 8	Cloudy	0		0	0	0	0	++	15 hr after last phage
3 10	Cloudy	0	0	+	0	0	0	++	3 cc in arm, 10 cc in bladder Be fore treatment
3 11	Cloudy	0	0	+	0	0	0	++	15 hr after last phage
3 14	Cloudy	0	0	0	0	0	0	++	3 cc in arm, 10 cc in bladder Be fore treatment
3 21	Cloudy	0	+	++	0	0	0	±	3 cc in arm, 10 cc in bladder Be fore treatment
3 27	Cloudy	0	0	0	0	0	0	0	3 cc in arm, 10 cc in bladder Be fore treatment
4 7	Clear	0	0	0	++			++	3 cc in arm, 10 cc in bladder
4 11	Clear							±	3 cc in arm, 10 cc in bladder
4 18	Clear	0	+	++					
5 29	Clear	0	±	+					3 cc in arm, 10 cc in bladder After 46 day absence
6 9	Clear	0	+	0	0	0	0	+	Filtrate active for Type 4 of Dec 27, 1929
6 16	Clear	0	+	0	0	0	0	0	3 cc in arm, 10 cc in bladder
6 20	Clear	0			0	0	0	0	Cultures of sixteenth still negative
6 21	Clear	0	+	0	0	0	0	0	3 cc in arm, 10 cc in bladder
6 27	Clear	0	++	0	0	0	0	±	3 cc in arm, 10 cc in bladder
7 3	Clear	0	±	0	0	0	0	+	3 cc in arm, 10 cc in bladder
7 11	Clear	0	0	0	0	0	0	+++	3 cc in arm, 10 cc in bladder
9 4	Clear	0			0	0	0	+++	2 months since last treatment
			<i>Interval of Four Months Since Last Treatment</i>						
11 7	Clear	0	0	0	0	0	0	0	3 cc in arm, 10 cc in bladder 4 months since last treatment
11 8	Clear	0	0	0	0	0	0	0	16 hours after phage
11 13	Clear	0	+	+	0	0	0	++	
			<i>Interval of Two Months Since Last Treatment</i>						
1931									
1 9	Clear	0	++	++	0	0	0	+++	3 cc in arm, 10 cc in bladder
1 10	Clear	0	0	0	0	0	0	0	
1 18	Clear	0	0	0	0	0	0	0	3 cc in arm, 10 cc in bladder
1 19	Clear	0	0	0	0	0	0	0	
1 23	Clear	0	0	0	0	0	0	±	3 cc in arm, 10 cc in bladder
1 24	Clear	0	0	0	0	0	0	0	

CASE 35 (Cont d)

DATE	CLFAR OR CLOUDY	ODOP	CFLLS	DIRECT BACT	CULTURE TYPES				CLINICAL NOTES
					1	2	3	4	
1931									
1 30	Clear	0	0	0	0	0	0	0	3 cc in arm, 10 cc in bladder
1 31	Clear	0	0	0	0	0	0	±	
2 6	Clear	0	0	0	0	0	0	++	3 cc in arm, 10 cc in bladder
2 7	Clear	0	0	0	0	0	0	±	
2 12									3 cc in arm, 10 cc in bladder
2 20	Clear	0	0	0	0	0	0	0	3 cc in arm, 10 cc in bladder Urine acid
2 21	Clear	0	0	0	0	0	0	0	
2 25	Clear	0	0	0	0	0	0	0	3 cc in arm, 10 cc in bladder
2 26	Clear	0	0	0	0	0	0	0	
3 6			±					++	
3 7								±	
3 20				0	0	0	0	0	
3 21				0	0	0	0	0	Urine alkaline
3 25				0	0	0	0	0	
3 26				0	0	0	0	0	73 observations to date

1932

1 6 Patient reports he is still symptom free and urine is odorless**

**1 27-32 after going to press Urine sample showed ++++ type A B coli No symptoms

CASE 36 — Ada S, aged two U H 220180 June 29, 1929 Patient brought to Hospital because of cleft palate when she was nine months old Had influenza at three months and frequent upper respiratory infections No complaints at present Examination aside from harelip negative

February 3, 1930 Returned for cleft palate During last two weeks has had a temperature as high as 104° Pneumonia was suspected Has some cough and some nasal discharge Examination negative except for evidence of undernutrition and the congenital defect

CASE 36

DATE	REACTION	CELLS	B COLI	CLINICAL NOTES
1930				
2 5		++++	+++	Alkalinization begun
2 11	alk	++++		
2 19		++		
2 27			+++	
3 11		++	+++	2 cc filtrate subcutaneously
3 19		++	+++	1 cc filtrate subcutaneously
3 21				Discharged
3 25				Returned Paracentesis right ear
3 31		++		Alkali discontinued
4 15		+	+++	
4 16		++		Cystoscoped Dilatation left kidney pelvis
4 25				Cystoscoped Dilatation right kidney pelvis
11 29		±	+++	Readmitted
12 3	acid			Alkalinization begun
12 12	acid			Increased alkali
12 26				Developed chickenpox
1 7		++	+++	Returned from Contagious Ward
1 14		++	+++	3 cc filtrate in arm, 7 cc in bladder
1 16			+++	3 cc filtrate in arm, 7 cc in bladder
1 18			+++	
3 17	acid	++	+++	3 cc filtrate in arm, 7 cc in bladder
4 1				Discharged We have not been able on constant effort to develop a filtrate completely lytic for patient's organism

February 5, 1930 Urine contained "pus" Catheterized specimen B coli +++ We were unsuccessful in developing a bacteriophage that would satisfactorily lyse the organism isolated from this patient's urine

CASE 37—Ida L, aged eighteen months U H 222282 July 29, 1929 Patient brought to the Hospital because of an unexplained fever Two months ago fever, vomiting, loose stools and anorexia developed Since this time she has had an afternoon elevation of temperature ranging between 102° and 105° She has had night sweats during the past week The mother thinks the patient has pain when she attempts to urinate

Examination Aside from a slight general adenopathy the physical examination was negative Urine acid, 50 to 70 leucocytes per low power uncentrifuged field, no casts, no blood, albumin negative There was a definite secondary anemia Hemoglobin 45 per cent Sahli Leucocytes 14,000 Kahn negative Alkalinization was begun The effect of bacteriophage treatment in this case was very satisfactory A suitable bacteriophage filtrate was developed in eight days

CASE 37

DATE	CELLS	B COLI	CLINICAL NOTES
1929			
7 27	+++	++++	
8 7	++	++++	Alkalinization begun July 29, 1931
8 14	++	++++	Hemoglobin 63 per cent Leucocytes 19,200
8 16	++	++++	2 c c filtrate subcutaneously in arm
8 17		++++	
8 19			2 c c filtrate subcutaneously in arm
8 20	0	+++	
8 21			2 c c filtrate subcutaneously in arm
8 22	0	+	
8 24	0	±	
8 26	0	±	Hemoglobin 65 per cent
8 28	0	0	
8 30	0	±	1 c c filtrate subcutaneously in arm
8 31	0	±	
9 4	0	±	
9 6	0	±	3 c c filtrate subcutaneously in arm
9 7	0	±	
9 9			25 c c whole blood intramuscularly
9 11			1 c c filtrate subcutaneously in arm
9 12		±	
9 26		±	25 c c whole blood intramuscularly
10 1			Discharged on sodium bicarbonate Return in 1 month
10 5	0	0	
10 6	0	0	
10 7	0	0	

CASE 38—Mrs D, aged fifty C H September 14, 1929 Patient operated upon for a small hemorrhoid and fissure in ano Ten days later she developed bladder and urethral irritation The urine showed no pus but on culture contained +++ B coli There was a history of similar attacks over a period of two or more years The bacteriophage findings are those of a long standing infection This patient recovered symptomatically after the fifth treatment but we were unable to sterilize the urine at any time A recent letter (December, 1931) from this patient reports she is symptom free

CASE 39—Lois S, aged seven C H October 28, 1929 The middle of June the patient contracted a "cold" which did not respond to treatment Since this time there have been frequent recurring temperature increases up to 100° F During the past three months she has been confined to bed because of the suspicion of tuberculosis Recently the increased temperature has occurred every day, and she has complained of "spasmodic, shooting, abdominal pain from the navel down" Sometimes the pain may last for half an hour It may be initiated by an evacuation of the bowels Her weight has remained stationary at

CASE 38

DATE	CELLS	B COLI	CLINICAL NOTES
1929			
9 24	±	+++	
10 12		+++	
10 16			3 cc filtrate in arm
10 17	+++	+++	
10 18			3 cc filtrate in arm
10 21			3 cc filtrate in arm
10 22	+++	++	
10 23			3 cc filtrate in arm
10 24	+	++	
10 30		±	
10 31			3 cc filtrate in arm
11 1	0	±	
11 3			3 cc filtrate in arm
11 5			3 cc filtrate in arm
11 6		++	
11 8		+	
11 11		+	
11 12		++	3 cc filtrate in arm
11 13		++	
1930			
4 28		+++	Large colony type No symptoms

51 pounds Sweating occurred for a short period in the beginning of the illness Recently urinary frequency and burning micturition developed

Physical examination was negative except for a marked accumulation of smegma and considerable irritation of the external genitals X ray examination of the lungs was negative A catheterized urine specimen showed no excess of cells, no casts, no abnormal chemistry At the end of twenty four hours on blood agar and Endos medium the culture was negative At the end of thirty six hours in brain heart infusion a positive growth of B coli was secured Alkalinization was begun It took many days (25) to adapt a bacteriophage for this organism Treatment was begun November 27, 1929

CASE 39

DATE	CELLS	B COLI	REMARKS
1929			
10 28	0	±	In brain heart infusion
10 30		±	
11 27	0	±	3 cc filtrate in arm
11 29	0	±	3 cc filtrate in arm
12 1			3 cc filtrate in arm
12 2	0	0	Culture still negative after 5 days
12 16	0	0	3 cc of filtrate in arm
12 17	0	0	18 hours after last treatment

The patient has been very much better Occasionally a slight temperature increase is found She has been having a mild upper respiratory infection that may be responsible

April 2 1930 Patient still continues to run an occasional temperature of 100° F She looks well, but has gained only half a pound There are no urinary symptoms or signs Culture negative This is a case of previously unexplained low fever associated with a resistant B Coli type of infection of the genitourinary tract which responded quite promptly to treatment after a prolonged effort to secure a satisfactory bacteriophage

Bacteriologic Note—October 28, 1929 Endos and blood agar plates negative in twenty four hours Brain heart infusion positive in thirty six hours Transplants to Endos and blood agar positive in twenty four hours, 14 colonies per standard loop These

were medium sized type R B coli colonies Hemolytic, translucent colonies on blood agar, metallic luster colonies on Endos

CASE 40—Mis S, aged thirty five C H October 29, 1929 Patient has had a colon bacillus infection of the urinary tract for many months Aside from a general indisposition and occasional local urinary irritation, the condition has not been marked It is desired to have the infection cleared up, as it may be a contributing factor to her feeling of indisposition This patient responded quickly to bacteriophage treatment

CASE 40

DATE	CELLS	B COLI	REMARKS
1929			
10 29	0	+	Alkalinization begun
11 27	0	+	3 cc filtrate in arm
11 29	0	±	3 cc filtrate in arm
12 2		0	3 cc filtrate in arm
12 16	0	0	3 cc filtrate in arm
12 18			15 cc filtrate in arm
12 19	0	0	2 cc filtrate in arm
12 20	0	0	
1930			
4 2	0	0	10 cc filtrate in bladder

April 2, 1930 The urinary symptoms had been very much improved until two weeks ago when there was a recurrence "Rheumatic" symptoms developed in the right loin at this time and continued On general principles 10 cc of the bacteriophage filtrate were injected into the bladder While doing this many small polypoid growths were seen in and around the meatus urinarius and on either side of the vagina posteriorly Culture of the urine was negative The symptoms were coming from the polypoid growths

CASE 41—Pearl K, aged ten U H 231448 January 5, 1930 Patient has been on Bone and Joint Service for twenty six days because of poliomyelitis residual palsies Developed scarlet fever today

CASE 41

DATE	CELLS	B COLI	CLINICAL FINDINGS
1930			
1 14	+++	+++	Alkalinization begun Temperature 103°
1 16	++	+++	Running a septic temperature, severe epistaxis
1 19			Mercles developing
1 20			Rash well out
1 21			Rash has subsided General condition improved
1 22			Unable to alkalinize urine Increase bicarbonate
1 25			Pain right ear, spontaneous rupture Right ear drum perforated Urine alkaline Marked cervical adenitis
1 26			Pain over left frontal and maxillary sinuses Left eyelids swollen
1 28			Colicky pains upper left abdomen General condition improved
1 30			Pain left ear Temperature normal
2 3			Fifth day of normal temperature
2 5	++	+++	Seventh day of normal temperature
2 8			Transferred from Contagious ward to Bone and Joint ward
2 20	+++	+++	Referred to Pediatrics for treatment
2 22	+++	+++	3 cc filtrate subcutaneously
2 23	0	+++	
2 24	0	+	
2 26	0	+	3 cc filtrate subcutaneously, 5 cc in bladder
2 27	0	++	5 cc filtrate in bladder
3 17		++	Case became lost to our service after this time
8 5	+++	+++	Returns for observation after three months' absence from Hospital Has been well Examination reveals septic tonsils, urine pus No albumin or casts Culture shows many B coli

January 12, 1930 Symptoms of scarlet fever have subsided

January 14, 1930 A fever of 101° developed today General examination negative Urine shows pus no casts, a large number of gram negative bacilli Alkalinization was begun Seven P.M. temperature 104.8° Face flushed This patient was not properly cared for Being on another service she was inadvertently overlooked There was a definite clearing up of the pyuria and a modification in the number of bacilli following treatment but this was not permanent

CASE 42—Louise H., aged two U H 246377 August 9, 1930 Patient brought to Hospital because of undernutrition Scarlet fever developed May 17, acute throat June 10 Throat was lanced Infected ears opened several times In Hospital four weeks Became rapidly emaciated after this

Examination Emaciated dehydrated slight rigidity of neck Leucocytes 19,600 82 per cent polys hemoglobin 70 per cent Spinal fluid clear under slightly increased pressure Fundi negative Guinea pig inoculated later negative Blood culture negative Transferred to Neurosurgery because of suspicion of brain abscess Ventricular puncture negative

August 22, 1930 Patient transferred back to Pediatrics

September 8, 1930 Improving Has gained 2½ pounds

September 11, 1930 Fever for past two days Urine contains pus and bacilli Culture started Alkalinization begun This patient made a satisfactory recovery

CASE 42

DATE	CELLS	B. COLI	CLINICAL NOTES
1930			
9 11	+++	++	Alkalinization begun Fever
9 19	±		Urine is almost clear Temperature normal all day
9 25	0		Much improved Leucocytes 14,000 Temp 100° Sitting up
10 1	+++		Measles Transferred to Contagious Hospital
10 14			Returned to ward
10 19	++++		Fever
10 24			Bacteriophage given
10 27			Bacteriophage given
10 28			Bacteriophage given
11 2		++	} Dose not recorded Probably 3 cc in arm, 7 cc in bladder
11 21	0	±	
11 26	0	0	
12 3			Discharged
1931			
1-4	0	0	Returned for check in the Out Patient Department Direct Smear— Advised to return in one month Patient had just recovered from chickenpox

CASE 43—Euphemia R., aged seven U H 253825 November 21 1930 Patient brought to Hospital because of attacks of "indigestion" which have been present at intervals for the past two years, irregular cramplike pains over the lower abdomen, nausea, occasional vomiting of food eaten at the previous meal fever The urine is said to have contained "pus" Her physician sends her to the Hospital for bacteriophage treatment

Examination—Well nourished child Temperature 100° Submaxillary and axillary glands palpable Otherwise a carefully done physical examination was negative The abdomen showed no abnormal reactions at any time Kahn negative Urine showed 15 to 20 cells per low power uncentrifuged field Culture showed B. coli — To be observed in Out Patient Clinic This patient improved symptomatically but the bacilluria did not clear up

CASE 43

DATE	CFLS	B	COLI	REACTION	CLINICAL NOTES
1930					
11 21	+	+++	acid	Urine is cloudy	Alkalinization started
12 11	+	+++	alk	Urine is cloudy	
12 17	+	+++	alk	3 cc filtrate in arm, 7 cc in bladder	Alk discontinued*
12 19	0	±	alk	3 cc filtrate in arm, 7 cc in bladder	
12 21	±	+++	acid	3 cc filtrate in arm, 7 cc in bladder	Alk resumed
12 23	0	0	alk	3 cc in arm, 7 cc in bladder	
12 26	0	0	alk	Urine has been water clear for several days	
1931					
2 26	++	+++	acid	Readmitted	Continues to have occasional attacks and vague lower abdominal pain, every 3 or 4 weeks with frequency GI X rays negative Alkalies begun
2 28	+	+++	acid	<i>Autogenous phage</i> found in urine	
3 1	+	+++			
3 4	+	+++	alk		
3 5	+	+++	alk	3 cc filtrate in arm, 5 cc in bladder	
3 7	+	+++	alk	3 cc filtrate in arm, 5 cc in bladder	
3 9			alk	3 cc filtrate in arm, 5 cc in bladder	
3 10	±	++	acid	Discharged on alkalies, to report later	
4 18				Readmitted	Well since discharge No pain Appetite good
4 20	±	+++	alk		
4 24	±	+++			
4 30				Discharged	Satisfactory filtrate has not been developed
6 8				Readmitted	Has had frequency, enuresis, abdominal pain, distention Urine said to have "pus" clumps
6 9	±	+++		<i>Autogenous phage</i> negative	
7 1				Alkalies begun	
7 2		+++			
7 10			alk	3 cc filtrate in arm, 5 cc in bladder	
7 15	+++	+++	alk	3 cc filtrate in arm, 5 cc in bladder	
7 17	±	+++	neut	3 cc filtrate in arm, 5 cc in bladder	
7 19	±	++			
7 21				Discharged	Much improved symptomatically Continue alkalies Return for further observation Patient has not returned (January 10, 1932)

*Alkalinization was begun November 21 1930 Through a misunderstanding the mother discontinued its use when filtrate injections were begun This was discovered December 21 when alkalinization was resumed and continued

CASE 44—Leola H, aged eight U H 258616 February 12, 1931 Brought to Hospital for "appendicitis" Complained of headache, abdominal pain at first to left of navel, now at McBurney's point, nausea, vomiting Said to have had an "abscessed kidney" three years ago

Examination—Tenderness at McBurney's point, with some rigidity Left costo vertebral tenderness Leucocytes 10,000 Urine 10 cells per low power field, an occasional granular cast Surgery suspects a subsiding appendicitis Referred to Pediatrics with thought that it may be an upper respiratory infection following a tonsillitis X ray of chest and our examination negative

February 20, 1931 Urine culture B coli +++ Few cells

February 24, 1931 Twelfth day in Hospital Tenderness over maxillary sinuses X ray showed maxillary and ethmoid infection Otology Department found no definite clinical evidence of sinus disease

May 15, 1931 Catheterized specimen of urine shows on culture a green producing streptococcus like organism having no B coli characteristics excepting that it was easily lysed by B coli bacteriophage filtrate

May 19, 1931 The same organism was recovered from patient's urine It was also easily lysed by Coli phage overnight When left alone it lysed itself in two days' time This made it impossible to keep a strain of this interesting organism as we had previously

CASE 44

DATE	CELLS	B. COLI	REACTION	CLINICAL NOTES
1931				
2 20	±	+++	acid	Alkalinization begun
2 22	±	+++	neut	
2 23	0	+	acid	Autophage found in urine
2 24	±	+++	acid	Autophage still present
2 25	±	+++	neut	Direct smear
	0	0	alk	Later in day Culture 0 Active phage present
2 26	++	++	neut	
2 27	±	+	alk	
3 1	±		alk	
3 3	±	++	alk	Autophage again present Being developed for treatment
3 4	±	++	alk	
3 5	±	+++	alk	3 cc filtrate in arm, 7 cc in bladder
3 7	±	++	alk	3 cc filtrate in arm, 7 cc in bladder
3 9				3 cc filtrate in arm, 7 cc in bladder
3 10	0	0	neut	Discharged
4 13	++			Returns to Out Patient Department Malaise, maxillary tenderness, septic tonsils
4 14				Discharged on acid treatment Not referred to laboratory
4 26	±			Returns in good condition Alkalinization resumed Sent home
5 15		+++		Returns Increased frequency, headache, nocturia, malaise Chills this A.M. Generalized abdominal tenderness No rigidity Costovertebral angle tenderness
5 19	++	+++		
6 3		0		Potent phage found in urine Direct smear showed organisms Disappeared from urine in 24 hours
6 9	±	0		Direct smear Streptococcus Reaction of autophage same as June 3 See text May 15, 1931

done with the original strain of B. coli. The lytic principle from this organism, this autolytic principle, was active against the original B. coli isolated from the patient's urine January 20, 1931.

CASE 45—Mrs. H. L. S., aged fifty. C. H. May 25, 1931. Patient has been having manifestations of pyelitis for many months, chills, urinary frequency and irritation coming on at intervals. Urotropin and other antiseptics have been used. A catheterized urine specimen showed slight excess of leucocytes, no casts, and on culture B. coli ++. Alkali-

CASE 45

DATE	CELLS	B. COLI	REMARKS
1931		P S	
5 25	-	- +++	Alkalinization begun*
6 19	0	- 0	Urine alkaline
7 6	0	± 0	3 cc filtrate in arm, 10 cc in bladder
7 7	0	± 0	3 cc filtrate in arm, 10 cc in bladder
7 8			3 cc filtrate in arm, 10 cc in bladder
7 9	0	± 0	
7 10	0	---	3 cc filtrate in arm, 10 cc in bladder
7 11			Urinary frequency, chills, irritation
7 13	0	±	Symptoms have disappeared
7 14	-	++	
7 20	-	+++	
7 21	0	0	Symptoms still absent
7 28	0	0	Symptoms still absent

*Bacteriology. The first culture showed no growth on Endos medium in twenty-four hours. Numerous very tiny green producing colonies resembling *Streptococcus viridans* developed on the blood agar plates. When these were transplanted in broth incubated and again transplanted on Endos typical B. coli R. type colonies developed. The tiny type of colony was never again encountered. It will be observed that alkalinization had been going on for twenty-five days and the urine was alkaline at the time of the second culture.

nization was begun This patient has remained free from symptoms to date (December 5, 1931)

CASE 46—Mrs C V, aged thirty one C H September 28, 1931 This patient has had a known colon infection of the urinary tract for over a year She has previously been treated in New York with a filtrate prepared by us She was very carefully watched by competent observers and responded fairly well to treatment The B coli, however, did not remain away from the urine At one time the culture is reported to have become negative She has had no symptoms since this time She presents herself for examination

The patient is a very robust person without any symptoms She comes in for a check up examination Aside from the bacilluria the examination was entirely negative The sequence of events follow

CASE 46

DATE	CELS	B COLI	URINE	REMARKS
1931				
9 28	0	±	acid	Alk administration begun
9 30	+	++++	acid	
10 13	±	++++	alk	3 cc filtrate in arm, 10 cc in bladder
10 14	0	±	alk	18 hours after first treatment
10 15	0	±	alk	3 cc filtrate in arm, 10 cc in bladder
10 16	0	0	alk	
10 17	0	0	alk	3 cc filtrate in arm, 10 cc in bladder
10 19	0	0	neut	3 cc filtrate in arm, 10 cc in bladder
10 21	0	+	alk	3 cc new filtrate in arm built from Oct 17 set up Fed 3 times a day It is much more potent
10 22	0	++	alk	19 hours after last treatment
10 23	0	±	alk	3 cc new filtrate in arm
10 24	0	±	alk	
10 26	0	++	alk	3 cc new filtrate in arm
10 27	0	+	alk	
10 29	±	+	alk	Organisms in the urine are agglutinated Heretofore they have always been free
10 31	0	±	alk	
11 2	0	±	neut	Allowed to leave city for a few days
11 12	±	++++	alk	Discharged on sodium bicarbonate and basic diet

This has been a very resistant strain of B coli It was definitely affected after the first series of injections We were however, never able to cause the organism to disappear from the urine for more than a few days A bacteriophage was not demonstrated in her urine at any time The patient is perfectly well and has been since her first course of treatment in New York She was discharged on alkaline treatment with the usual advice

CASE 47—Mrs F D G, aged sixty six C H December 31, 1931 Last summer (August) the patient had symptoms attributed to a cystocele bearing down sensation interfering with her walking Replacement and douching overcame the symptoms A ring pessary was introduced in October This caused much discomfort in the rectum and had to be abandoned Another pessary was tried Three days later bladder symptoms developed, painful micturition and urinary frequency, day and night This was treated for several days with bladder irrigations She has taken urotropin since October, but only five grains every morning Some other urinary antiseptic was used, possibly not more adequately than the urotropin Recently she has been taking soda For the past seven years she has taken more or less soda for "gastric ulcer" On entrance urinary frequency and painful urination were still present

Examination showed a fairly well preserved woman for her years Glaucoma Thin edge of liver felt two fingers below the costal margin No nodules A few days after the bladder discomfort began she complained of generalized pain in the abdomen and a slight increase in temperature which lasted for five days Tenderness on pressure over the abdomen at this time, not localized The perineum is inadequate There is a rectocele and a cystocele There is no redness about the urethra The uterus is normally atrophied

A catheterized urine specimen showed 150 cells per low power uncentrifuged field with clump. The culture showed +++ B coli

CASE 47

DATE	CFLLS	B COLI	PFM APKs
1931			
12 31	—	—	Alkalinization continued
1932			
1 6	+	—	Urine alkaline 3 c c bacteriophage in arm 10 c c in bladder
1 8	—	—	Urine alkaline 3 c c bacteriophage in arm 10 c c in bladder
1 11	+	0	Urine alkaline 3 c c bacteriophage in arm 10 c c in bladder
1 13	0	0	Urine alkaline 3 c c bacteriophage in arm 10 c c in bladder

Patient's symptoms have all disappeared

METHODS USED FOR DEVELOPING BACTERIOPHAGE FOR TREATMENT OF B COLI INFECTIONS

By the use of our sewage base method* many days time is saved over other methods of bacteriophage development which we have tried. It is possible to develop a water clear filtrate which gives only a faint pink local reaction at the site of inoculation without the occurrence of edema or a general reaction. Filtrates developed in broth without subsequent development on solid media give marked reactions. Filtrates entirely or finally developed on solid media give only slight reactions. The methods in use at the present time are as follows:

Sewage Base—Sewer water is filtered clear through gauze paper and infusorial earth. To 100 c c of the resulting filtrate 0.4 gram of dehydrated bactonutrient broth (Difco) is added. After thoroughly mixing this is sterilized by filtering through a Berkefeld M candle.

Selecting a Bacteriophage—To twelve sterile test tubes approximately 8 c c of the sewage base is added. One of these is used for a control. The others are fed with varying amounts of a nine-hour culture of the clinical strain of B coli. One tube is filled with broth as a control. Between the ninth to the fifteenth hour these tubes must be frequently and carefully watched for it has been our observation that the bacteriophage under these conditions does not

TABLE IV

TUBE NO	CHARACTER OF TUBE	GROWTH RESULT AFTER 9 TO 15 HOURS INCUBATION
1	8 c c broth control + 1 mm loop B coli suspension	—
2	8 c c filtrate control	0
3	8 c c filtrate + 1 mm loop 9 hr B coli suspension	+
4	8 c c filtrate	—
5	8 c c filtrate	—
6	8 c c filtrate	0
7	8 c c filtrate	—
8	8 c c filtrate	—
9	8 c c filtrate	—
10	8 c c filtrate	0
11	8 c c filtrate	0
12	8 c c filtrate	—

*Cowie D M. Observations on the Bacteriophage. Ann Clin Med 1 73 1926
 Cowie D M. The Present Status of the Bacteriophage in Colon Infections of the
 K L L tract Transactions of U of M Med and Infect Dis Society 1929

develop in appreciable amount until after nine hours, and that secondary growth of resistant organisms may start as early as the fifteenth hour, or some contaminating organism may render the tubes cloudy, thus obscuring lysis that may have occurred. A set up and results may be illustrated as shown in Table IV.

Building the Stock Phage Filtrate—Add all the negative and one plus filtrates together (3, 6, 8, 10, 11, 12). In this instance it will make a batch of 48 c.c. Filter through a Berkefeld M candle to render sterile. It will be seen that each of the tubes selected was able to lyse to all practical purposes, one loop of the nine-hour broth culture. In other words, the combined filtrates had lysed six loops. It will be safe then to inoculate the combined filtrate with 3 loops of the nine-hour broth culture. After nine hours incubation we usually get a clear fluid.

Feed the resulting fluid with the same amount of culture without filtering if it is clear, otherwise filter and feed. Incubate nine hours as before.

On successive nine-hour feeds gradually increase to 3, 5, 6, 9 loops, and then to 1 c.c. of the culture, repeating each successive increase once. In this way if great care is taken not to feed too much, subsequent filtration may be avoided and much time saved. The point is to feed just enough to prevent visible growth.

This builds our stock of active concentrated bacteriophage filtrate from which we build our individual filtrates for treatment. We keep these stock filtrates constantly developing. Usually nine or ten are always on hand, each one of which may be tested against a patient's strain on agar plates which shows plainly and most easily whether the filtrate is active for the patient's strain. This information is obtained in from eight to fifteen hours.

Building the Treatment Filtrate—This may be done in one of three ways.

First Method Seed one to three plain agar 6 inch plates with two drops of a nine-hour broth growth of the patient's strain. Dry in the incubator for three quarters of an hour. Cover with two or three drops of the bacteriophage filtrate. Incubate for nine to fifteen hours. Wash off the organisms with about 12 c.c. of sterile saline solution with the aid of a smooth bent glass rod, and pipette into plate No. 2. Wash and in like manner pipette into plate No. 3 using sterile technique throughout. Now pipette the contents of the third plate on to a sterile fourth plain agar plate and incubate all the plates for varying lengths of time, until satisfactory lysis is observed (nine to fifteen hours).

It is generally found that plates 1, 2, and 3 will be covered with growth and plaques, the plaques predominating, while plate 4 will look the same as it did when it was put into the incubator. That is, the added combined suspension has evaporated very little, and the organisms present have settled to the bottom, giving the appearance of a smooth yellowish white layer on the surface of the agar.

Plates 1, 2, 3, and 4 are now washed a second time with 20 to 30 c.c. of sterile saline solution. The resulting combined mixture is centrifuged until clear. The supernatant fluid is removed, sterilized by passing through a Berkefeld M candle, cultured for sterility and tested for bacteriophage activity by one of the following methods.

(a) By testing the action of varying amounts of filtrate against a nine hour broth suspension of the clinical strain. This may be done in the following manner. In each of five small, 2-inch test tubes place 10 drops of the broth suspension and serially 1 2 3 4 and 5 drops of the bacteriophage filtrate. The tubes are then put in the incubator and carefully watched between the ninth and twentieth hours. The tubes having the largest amounts of the filtrate are more likely to be found clear. However we not infrequently find a clear tube among the weaker dilutions. This fact has been demonstrated by D Herelle.

(b) By adding $\frac{1}{2}$ c.c. of the filtrate to varying quantities of a nine-hour suspension of the clinical strain 1 2, 3 4 5 and 6 c.c. respectively. These preparations are incubated and observed in the manner previously described. The tube showing the greatest lyses is accepted as demonstrating the lytic power of the bacteriophage.

(c) By seeding a 3-inch agar plate with 1 to 3 drops of a nine-hour broth suspension of the clinical strain and diving in the incubator for three-quarters of an hour. A square is marked on the bottom of the Petri dish with a wax pencil or a pen. This is the test area. The margins serve as control areas. Spread carefully over the square area one or two drops of the filtrate to be tested and observe in the incubator from the ninth to the twenty-fourth hour. Between these hours it will usually be found that from a few plaques to a complete lysis or a complete inhibition of the growth in the square has been effected while a luxuriant growth has developed over the control margins of the plate.

Second Method One hundred to 500 c.c. of the first bacteriophage filtrate are placed in a sterile Erlenmeyer flask. To this is added 1 to 5 c.c. of a nine-hour broth culture of the patient's strain. This mixture is allowed to stand at room temperature for from three to seven days at which time a heavy growth will be found unless a very potent bacteriophage has developed. This mixture is sterilized by filtration through a Berkefeld M candle. The P_H is corrected to 7.8. The growing factors are again added (0.4 gm. dehydrated bactonutrient broth—Difco) and the fluid is again inoculated with 1 to 5 c.c. of a nine-hour broth suspension of the clinical strain, incubated for three to seven days, and filtered. The P_H is corrected and this procedure repeated until no further growth results and the fluid remains clear. It is then tested for bacteriophage activity against the clinical strain by the means described under the first method.

Third Method Take 100 c.c. of the filtrate, add one loop of a broth suspension of the clinical strain incubate nine hours. The contents of the flask should be clear because purposely only a small feeding has been given. The flask is now fed three times a day. On the first day one drop at each feeding, on the second day three drops, on the third day five drops, on the fourth day ten drops. This develops the potency high enough to test for its therapeutic activity as previously described. The idea of this method is to present to the developing bacteriophage a task it can easily handle in the time allotted, in other words, assuring that the bacteriophage will never be overwhelmed by too large numbers of *B. coli*. These filtrates may be developed so high that they will stand high dilution or they may be developed once or twice more on solid media thus eliminating the great excess of protein.

Comment on the Methods Presented—The first method eliminates nutrient broth from the final product. The process of washing if carefully done need remove very little of the hard culture media. The degree of dilution seems sufficient to prevent unpleasant reactions in the large percentage of patients. The second method necessarily furnishes a filtrate containing considerable protein, which can be reduced only by diluting the resulting filtrate. Oftentimes the lytic power is great enough to stand diluting sufficiently to overcome undesirable reactions. Great care has to be taken to keep the P_{H} of this filtrate between 7.4 and 7.6. The filtrate secured by the third method also contains considerable protein. We have felt that by the frequent feeding the lytic principle has often developed more rapidly. For all purposes we think that methods utilizing hard media are preferable because they are less likely to contain substances that may cause reactions and because we are more correct in interpreting beneficial results as being due to the bacteriophage.

CONCLUSIONS

1 Bacteriophage inoculation in colon infections of the urinary passages is an effective method of treatment.

2 Success in treatment with bacteriophage depends upon careful individual adaptation of the bacteriophage corpuscles to the strain or strains of *B. coli* responsible for the infection, and careful previous preparation of the patient by alkalization and a continuance of alkalization for some time after the urine has become sterile.

3 The comfort of the patient following bacteriophage inoculation depends on reducing the protein content of the filtrate to the minimum. Water clear filtrates produce little or no reaction. Colored filtrates almost invariably produce undesirable reactions which seem more likely to occur in adults than in young children.

4 Recent acute *B. coli* infections are usually quite promptly terminated by bacteriophage inoculation.

5 Chronic *B. coli* infections are more resistant to the action of the bacteriophage. It is often more difficult to develop a satisfactory filtrate for this type of infection.

6 One course of *B. coli* bacteriophage inoculations does not prevent subsequent inoculation being effective.

7 Very often long standing infections may be terminated or greatly improved by bacteriophage inoculation.

One is impressed with the feeling that if a bacteriophage can finally be developed that will properly lyse the organism *in vitro*, sterilization will occur no matter how resistant the strain or how long standing the infection.

9 It appears that no immunity to subsequent attacks is conferred by bacteriophage sterilization of the urinary tract.

The bibliography on the bacteriophage is comprehensively brought up to date in Dr D'Herelle's books, published by Williams & Wilkins Company. The senior author wishes to express appreciation of the very careful assistance of Mr. Henry Poncher, Mr. Robert Hicks and Mr. Elmer De Gown at various times during the progress of these observations.

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RESPIRATION OF MICROORGANISMS*†

F G NOVY ANN ARBOR, MICH

RESPIRATION is a fundamental characteristic of life. It is the source of energy which a cell must have in order to carry on its complex activities. The study of cell metabolism may be directed and usually is toward the kind and amount of intermediate or of final cleavage products resulting from the utilization of carbohydrates, fats, proteins or other complexes. It may concern the action and nature of the diverse enzymes made by the organism or it may deal with the physicochemical conditions within and without the cell which underlie all functional manifestations.

Every living cell respire whether it leads an independent existence or whether it is grouped with like cells to form the tissues and organs of higher plants or animals. The chief product is the gaseous CO_2 irrespective as to whether the given organism lives in the presence or absence of oxygen. To Lavoisier respiration was a combustion process in which molecular oxygen united with organic matter. It may still be so considered although the process itself is by no means as simple as this would imply.

For the study of the gas exchange or respiration of microorganisms two main procedures are available. In one a relatively large volume of gas is analyzed and for this purpose the gas buret in some form is used preferably of the Henderson-Haldane type. In the other which is essentially microchemical the manometer of Barcroft is employed. The latter of comparatively recent origin has been used to determine the respiration of tissues as well as that of unicellular organisms such as yeast, bacteria, and protozoa.

The buret method is to be preferred whenever it is desired to study the respiration of a microorganism under varying conditions as regards the com-

*From the University of Michigan

†George W. Kober Foundation Lecture delivered at Gaston Hall Georgetown University Washington D C March 28 1931

position of the medium and the influence of varying concentrations of O_2 , CO_2 or other gases. In short it is applicable to the actual laboratory conditions under which a given culture is growing. The test tube culture is the one which is commonly used in the laboratory and for that reason it is highly desirable to have definite data as to the relative and absolute change that takes place within a tube.

The determination of the gaseous CO_2 in the air above the medium does not give the total amount of CO_2 formed. It is a well known fact that some organisms produce a considerable amount of basic products and, if such is the case some of the CO_2 will be fixed by the alkali. The estimation of this dissolved CO_2 requires a special procedure. The amount of CO_2 thus held in solution at times may be considerable. It may vary from practically zero to as much as 25 or 30 per cent of the total CO_2 . The very low respiratory quotients often obtained with the Barcroft manometer and in other methods where the dissolved CO_2 is neglected may thus in part, be accounted for.

A complete analysis, which includes the estimation of dissolved CO_2 , involves the destruction of the organisms and for that reason it is not possible to have a count of the number present in the culture. A very rough approximation of that number could be made by counting the number present in a control culture grown under like conditions. Similarly, it would be possible to obtain the weight of the organisms in a control tube. Neither procedure was attempted.

For the reason given it was not possible to determine the extent to which the glycerine, glucose or other constituents of the medium were utilized during the respiratory period. The data sought and obtained represent therefore merely the gas exchange effected by the growth of a given organism on a fairly uniform surface area (15 to 18 sq. cm.)

The methods employed in this investigation have been fully described elsewhere.* It was necessary for this study to devise a manometer which would indicate the pressure changes taking place in the culture tube or jar. When it is desired to make an analysis, the manometer with its attached tube or jar is placed on a suitable platform and a connection is established with the gas buret. After displacement of the air in the connecting tube, with mercury, the gas in the culture tube is drawn into the buret and analyzed.

When this analysis has been made, the culture tube is taken out and aerated after which the dissolved CO_2 is determined. The sum of the gaseous and dissolved CO_2 represents the total CO_2 produced in a given experiment.

Carbonic Acid Production—The amount and rate of production of this gas, under constant temperature conditions, varies with the organism and with the composition of the medium. Given the presence of a readily utilizable substance it is to be expected that multiplication of the organisms will be increased, and hence, an increased rate and intensity of the respiratory process.

Some data for the human tubercle bacillus are given in Table I. This organism does not grow on plain nutrient agar, and hence, the gas exchange is nil. On serum agar there is a fair growth in twenty-eight days with the

*VOIG, F. G., Roehm, H. R. and Soule, M. H. J. Infect. Dis. 36: 109-167, 1925.

TABLE I
GAS EXCHANGE PER TUBE OF HUMAN TUBERCLE BACILLUS GROWN AT 37° C
C C AT 0°, 760 MM

	NO DAYS	CO MADE	O LOST	CORRESPONDING AIR VOLUME AT 37° C
Plain Agar		0	0	
Glycerol Agar	12	48	57	334
	27	124	149	874
Glucose Agar	26	26	25	149
	85	66	70	410
Serum Agar	28	21	23	135

production of 21 c c of CO₂. The organism in this case is utilizing protein matter. When glucose is added to the agar the organism avails itself of the carbohydrate with the result that more CO₂ is produced than on the serum agar and the growth itself is better.

The addition of glycerine to agar or potato is known to be beneficial in promoting the growth of the tubercle bacillus. It was originally employed with the view of preventing the desiccation of the medium during the long period of incubation. As a matter of fact revealed by gas analysis the advantage derived from glycerine lies in its ready utilization by the organism as a source of energy. It will be seen that the tubercle bacillus growing on this medium produced 124 c c of CO₂ in twenty-seven days or 5 to 6 times as much as on glucose or serum agar.

For comparison, the gas exchange of the bovine tubercle bacillus is given in Table II. Here it will be seen that this organism grows about equally well on plain agar and on serum agar. The amount of CO₂ produced at the end of twenty-eight and fifty-six days is approximately the same for the two mediums. By contrast, with the human tubercle bacillus the bovine strain on the glucose medium gave in twenty-eight days a much higher yield of CO₂, 104 c c as against 26 c c. Noteworthy is the fact that this represents the maximum development for on further incubation only a slight increase in CO₂ resulted (108 c c). The dissolved CO₂ at this point amounted to 7 c c.

TABLE II
GAS EXCHANGE PER TUBE OF BOVINE TUBERCLE BACILLUS GROWN AT 37° C
C C AT 0°, 760 MM

	NO DAYS	CO MADE	O LOST	CORRESPONDING AIR VOLUME AT 37° C
Plain Agar	28	21	22	118
	56	24	29	170
Glycerol Agar	28	89	100	586
	56	155	174	1020
Glucose Agar	28	104	98	579
	56	108	105	616
Serum Agar	28	19	22	118
	56	28	33	193

showing the presence of an appreciable amount of alkali. Other oxidation products undoubtedly, contributed to bring about cessation of growth. On glycerine agar, although at the end of twenty-eight days the production of CO_2 was somewhat less than on glucose agar, no inhibition of respiration took place on further incubation, and consequently at the end of fifty-six days, the culture produced 155 cc of CO_2 as compared with 108 cc on the glucose medium. As in the case of the human tubercle bacillus, the glycerine agar gave the best growths and the maximum gas exchange.

Table III presents a summary of the results obtained with the diphtheria bacillus. The CO_2 production at the end of seven and twenty-eight days was distinctly higher with glycerine agar than with the other mediums. Even more striking is the fact that while the production of CO_2 on the plain and

TABLE III

GAS EXCHANGE PER TUBE OF DIPHTHERIA BACILLUS GROWN AT 37°C C.C. AT 0° , 760 MM

	NO DAYS	CO MADE	O LOST	CORRESPONDING AIR VOLUME AT 37°C
Plain Agar	7	16	17	99
	28	19	22	129
Glycerol Agar	7	21	26	152
	21	27	33	193
Glucose Agar	7	6	7	41
	28	20	23	134
Blood agar	7	18	19	111
	28	20	22	129

on serum agar, at the end of twenty-eight days, was about the same as that of the bovine tubercle bacillus, on glycerine and on glucose agar it was greatly inferior. Thus, on glucose agar only 20 cc of CO_2 were produced, while the bovine tubercle bacillus produced 108 cc. The maximum growth of the diphtheria bacillus is attained in a shorter time, and hence respiration is retarded early. Compared with the tubercle bacillus a relatively small amount of energy is needed. To what extent inhibiting substances play a part in this cessation of growth is not known.

TABLE IV

GAS EXCHANGE PER TUBE OF GLANDERS BACILLUS GROWN AT 37°C C.C. AT 0° , 760 MM

	NO DAYS	CO MADE	O LOST	CORRESPONDING AIR VOLUME AT 37°C
Plain Agar	7	17	21	123
	21	21	25	146
Glycerol Agar	7	88	102	598
	21	141	164	962
Glucose Agar	7	54	55	322
	21	122	126	739
Blood agar	7	38	45	264
	21	38	44	258

The results obtained with the glanders bacillus are given in Table IV. This particular strain grew fairly well on plain agar and somewhat better on blood agar. On blood agar the maximum CO_2 production was obtained in about seven days (38 c.c.) since there was no further increase at the end of twenty-one days. On glucose agar the CO_2 production rose from 54 c.c. in seven days to 122 c.c. in twenty-one days. With glycerine agar the gas exchange was even more pronounced for the yield of CO_2 was 88 c.c. at the end of seven days and 141 c.c. in twenty-one days. The utilization of glucose and of glycerine here was more pronounced than in the case of the tubercle bacillus.

The results obtained with a nonpathogenic organism the hay bacillus, are given in Table V. It might be expected that a saprophyte would be able to utilize freely proteins as well as carbohydrates. This is actually the case, since

TABLE V
GAS EXCHANGE PER TURE OF HAY BACILLUS GROWN AT 32° C C.C. AT 0°, 760 MM

	NO. DAYS	CO. MADE	O. LOST	CORRESPONDING AIR VOLUME AT 32° C
Plain Agar	7	66	72	409
	56	84	85	483
Glycerol Agar	7	76	89	505
	20	205	244	1386
Glucose Agar	7	74	62	352
Serum Agar	7	65	75	426
	21	142	159	903

in seven days the yield of CO_2 on the four mediums was 65 to 76 c.c. which is considerably higher than the yield obtained with the other organisms mentioned, with the one exception of the glanders bacillus on glycerine agar. At the end of twenty days the glycerine agar culture of the hay bacillus yielded 205 c.c. of CO_2 which is an unusual value.

The results obtained with the five bacterial organisms could be extended by the addition of others. They serve however to show that these organisms carry on an active respiration and yield a considerable though variable amount of CO_2 . In this respect they differ in no wise from typical animal organisms the protozoa. In Table VI will be found some of the results obtained with *Trypanosome*, *Leishmania* and *Herpetomonas* cultures. These organisms were grown on glucose blood agar and for that reason the values obtained are not directly comparable with those given in the preceding tables. It will be seen however, that with one exception these protozoa produced 60 to 70 or more c.c. of CO_2 in twenty-one to twenty-eight days. The slight irregularity may in part be due to uneven inoculation of the surface of the medium. This yield of CO_2 was greater than that obtained with the human tubercle bacillus and the diphtheria bacillus on glucose agar.

That the unicellular bacteria and protozoa respire is evident from the data presented. In this respect they behave exactly the same as the cells in the complex animal body. Plant tissues, however are no exception to the

TABLE VI

GAS EXCHANGE PER TUBE OF SOME PROTOZOA GROWN IN JARS AT 34° C C C AT 0° C, 760 MM, MOIST, GLUCOSE BLOOD AGAR

ORGANISM	NO DAYS	CO ₂ MADE	O LOST	CORRESPONDING AIR VOLUME AT 34° C
Tr Lewisii	21	59	63	352
L tropica	21	59	63	352
L Donovanii	28	62	65	363
L infantum	28	29	29	162
H oncopelti	28	72	75	419
H culicidarum*	28	76	78	435
H culicidarum**	28	73	76	424
H hyaerorum	28	58	61	340
H media	28	60	63	352
H muscidarum	28	62	64	357
H parva	28	58	62	345

*From *Culex pipiens***From *Anopheles quadrimaculatus*

rule as has been shown by many investigators. As an example may be mentioned the respiratory change carried on by the potato. The results of two experiments are given in Table VII. In these tests strictly sterile cylinders of

TABLE VII

GAS EXCHANGE PER 10 GRAMS OF RAW POTATO AT 34° C C C AT 0°, 760 MM

NO DAYS	CO ₂ MADE	O LOST	CORRESPONDING AIR VOLUME AT 34° C
3	70	69	396
10	94	96	552

raw potato were placed in tubes which were connected with manometers in the same way as the in case of cultures. Analyses made at the end of three and ten days gave 70 and 94 cc of CO₂, respectively, calculated per 10 grams of potato. It may be added that as long as oxygen is present the potato respire the same as an aerobic organism. When the oxygen has been consumed it continues to respire but as an anaerobe.

Oxygen Consumption—The production of CO₂ by a cell implies the utilization of oxygen regardless of whether that element is derived from the air or from compounds rich in oxygen. In the case of the aerobic organism it is the free oxygen of the air which is being consumed.

A cursory inspection of Tables I to VI will show that, as a rule, the volume of oxygen lost or rather consumed is somewhat larger than that of the CO₂

produced. The difference between the two volumes does not indicate that assimilation or retention of oxygen by the organism has taken place. It merely means that in addition to oxidizing carbon the oxygen has been used to burn hydrogen to water. It might even be used to oxidize the nitrogen or sulphur present in the protein which is being consumed. Oxygen consumption in excess of CO_2 production occurs when protein or glycerine is being utilized.

In the oxidation of a carbohydrate such as glucose $\text{C}_6\text{H}_{12}\text{O}_6$, the oxygen consumption should equal the CO_2 production. The values found when the organisms are grown on glucose agar or glucose blood closely approximate theory. They are usually a trifle higher and this indicates that the organism is chiefly oxidizing glucose and only to a slight extent protein matter.

It is noteworthy that the largest consumption of oxygen occurs when these organisms are grown on glycerine agar. This is to be expected considering the large amount of CO_2 produced. It merely means that glycerine is more readily utilized as a source of energy than are the carbohydrate or protein constituents of the medium.

The extent to which a given substance such as glycerine is utilized varies with different organisms. This fact can be made use of in differentiating between closely allied species. Thus the oxygen consumption of *B. melitensis* when grown on glycerine agar is about two-thirds that of bovine *B. abortus* and less than one-half that of the porcine strain of *B. abortus*. A somewhat similar difference in the oxygen consumption of these three organisms was observed when they were grown on glucose agar.

Air Volume—Without analytical data no inference can be drawn as to the volume of air which must be supplied to an organism in order to obtain a good growth. In general it has been supposed that there was enough oxygen in the air of a culture tube to meet its needs. The failure to realize that some organisms require a very large volume of air has led to false conclusions. Referring to Table I it will be seen that the tubercle bacillus at the end of twelve days, at which time only a slight growth had formed on glycerine agar had consumed 57 c.c. of O_2 which, at 37°C corresponds to 334 c.c. of air. Obviously, no growth of this organism would be obtained in a sealed or stoppered tube of 20 c.c. capacity. To obtain a rich growth of this organism such as that given in twenty-seven days a volume of air corresponding to 874 c.c. must be made available. The relatively poorer growths on serum or glucose agar exhausted the oxygen from 135 and 149 c.c. of air. It is evident therefore that in the cultivation of the tubercle bacillus, whether it be the human or the bovine strain, a ready and free access of air must be provided. A pinhole through the sealing wax or paraffin used to seal the tube is sufficient for this purpose.

The relatively low consumption of O_2 by the diphtheria bacillus (Table III) is reflected in the corresponding volume of air. Thus on glycerine agar at the end of twenty-one days the organism exhausted the oxygen from 193 c.c. of air which is a larger volume than was drawn upon by the culture on the other mediums. On the other hand the glanders bacillus in the same time utilized the oxygen out of 962 c.c. of air.

The largest consumption of oxygen was noted in connection with the hay bacillus (Table V) when grown on glycerine agar. The 244 cc of O_2 lost corresponds to an air volume of 1386 cc at $32^\circ C$. The avidity of this organism for air is in keeping with its saprophytic nature.

The protozoa, as shown in Table VI, when grown on glucose blood agar consume a fairly large amount of oxygen 61 to 78 cc, which corresponds to an air volume of 340 to 435 cc. This is three times as much as the air volume utilized by the diphtheria bacillus when grown on glucose agar.

Respiratory Quotient—This value is obtained by dividing the total volume of CO_2 produced by that of the O_2 consumed. The quotients calculated from the analytical data given in Tables I to V are assembled for convenience in Table VIII. It should be mentioned that in the tables the decimals of a cubic

TABLE VIII
RESPIRATORY QUOTIENTS FOR TABLES I TO V

	GLYCERINE AGAR	GLUCOSE AGAR	PLAIN AGAR	SERUM AGAR
Human Tubercle B	(12) 0.83 (27) 0.84	(26) 1.04 (85) 0.94		(28) 0.91
Bovine Tubercle B	(28) 0.89 (56) 0.89	(28) 1.06 (56) 1.03	(28) 0.95 (56) 0.83	(28) 0.86 (56) 0.85
Diphtheria B	(7) 0.81 (21) 0.82	(7) 0.86 (28) 0.87	(7) 0.94 (28) 0.86	(7) 0.94 (28) 0.91
Glanders B	(7) 0.86 (21) 0.86	(7) 0.98 (21) 0.97	(7) 0.81 (21) 0.84	(7) 0.84 (21) 0.86
Hay B	(7) 0.85 (20) 0.84	(7) 1.19	(7) 0.91 (86) 0.88	(7) 0.86 (21) 0.89

*The figures in parenthesis indicate the age of the culture in days.

centimeter were omitted and consequently the quotients given in Table VIII are only approximate. They are, however, sufficiently accurate for the purpose of comparison.

If an organism growing on a given medium such as glycerine agar, consumed only glycerine then the respiratory quotient should correspond to the theoretical value of 0.857. Similarly, with glucose agar the value would be 1.0. It is obvious, however, that an organism while utilizing such substances as a source of energy must also make use of other nutrient compounds such as proteins and amino acids. The result is that the observed quotient may be somewhat higher or lower than the expected theoretical value.

It will be seen from Table VIII that the quotients for the five organisms, grown on glycerine agar, approximate the theoretical value of 0.85. Similarly, the quotients for the cultures grown on glucose agar approach the value of 1.0. The most marked discrepancy is that presented by the diphtheria bacillus and this would indicate that it makes relatively very little use of glucose. The gas exchange (Table III) due to this organism whether grown on plain agar, glucose agar, or blood agar is much the same.

The quotients obtained for the cultures on plain agar and on serum agar are appreciably higher than the theoretical quotient for protein. It must be

assumed therefore that other substances having a higher quotient than 0.81 are undergoing oxidation

Deserving of note is the fact that young and old cultures of a given organism have essentially the same respiratory quotient. The ratio of gas exchange, as might be expected, is the same for a small number of young cells as for the greatly increased number represented by an old culture. Conceivably, with old cultures decarboxylation could be expected as a secondary reaction, in which case the quotient would be higher than at the beginning.

In Tables IX, X and XI are given average values for the respiratory quotients of these organisms when grown on the different mediums. The number

TABLE IX
AVERAGE OF CORRECTED REAL RESPIRATORY QUOTIENTS OBTAINED IN JAP EXPERIMENTS*

MEDIUM	THEORY	TUBERCLE BACILLI	
		HUMAN	BOVINE
Plain agar	0.81		0.888 (4)
Glycerol agar	0.857	0.856 (13)	0.903 (9)
Glucose agar	1.0	0.992 (3)	1.036 (3)
Serum agar	0.81	0.904 (3)	0.852 (4)

*The figures in parenthesis give the number of experiments

TABLE X
AVERAGE OF CORRECTED REAL RESPIRATORY QUOTIENTS OBTAINED IN JAP EXPERIMENTS*

MEDIUM	THEORY	DIPHTHERIA B	GLANDERS B	HAY B
Plain agar	0.81	0.921 (4)	0.841 (5)	0.912 (10)
Glycerol agar	0.857	0.802 (4)	0.859 (4)	0.843 (8)
Glucose agar	1.0	0.906 (4)	0.972 (4)	1.278 (9)
Serum agar	0.81	0.942 (4)†	0.848 (4)†	0.874 (8)
Potato	1.0		1.044 (4)	

*The figures in parenthesis give the number of experiments

†Blood agar used

TABLE XI
AVERAGE OF CORRECTED REAL RESPIRATORY QUOTIENTS OBTAINED IN JAP EXPERIMENTS
PROTOZOA AND POTATO*

MEDIUM	TR. LEWISI	L. TROPICA	L. INFANTUM	L. DONOVANI
Blood agar	0.822 (4)	0.875 (4)	0.868 (4)	0.862 (4)
Glycerol Blood agar			0.791 (2)	0.832 (2)
Glucose Blood agar	0.938 (3)	0.951 (3)	1.002 (4)	0.968 (4)
R Q of Potato	Theory 1.0, found 1.006 (3)			

*The figures in parenthesis give the number of experiments

in parenthesis following each quotient indicates the number of experiments made and averaged. These average respiratory quotients can be considered as fairly accurate values for the given organisms when grown on the stated mediums.

Varying Oxygen Concentrations—When an agar tube inoculated with an aerobic organism is placed in a small jar which is attached to a manometer it will be evident before long that growth and respiratory changes are taking place. The growth continues until the manometer shows a constant reading at which point the oxygen content of the confined air volume is reduced to zero. It follows therefore that the organism, starting with 20.9 per cent of oxygen has been growing in progressively decreasing concentrations of that gas. An experiment such as this does not reveal the effect, if any, of varying tensions of oxygen on the inoculum itself. This can only be ascertained by placing each one of several tubes in a separate jar into which a definite volume of oxygen is introduced. An essential condition for the success of a comparative experiment of this kind is that the absolute volume of oxygen necessary for good growth shall be practically the same in each container. This requirement will necessitate increasing the size of the container with each decreasing tension. It has been shown that to obtain a good growth of the tubercle bacillus, about 100 c.c. of oxygen must be made available. If, therefore, it is desired to have concentrations of oxygen corresponding to 5, 3, 1, and 0.5 per cent it will be necessary to use containers of 2, 3.3, 10, and 20 liters capacity to supply the requisite 100 c.c. of oxygen.

Experiments made with the tubercle bacillus under conditions approximating those just given revealed the interesting fact that the growth of the organism was not proportional to the amount of O_2 consumed. Since each container held about 100 c.c. of O_2 and all of this was consumed, it could be expected that the growth would be quantitatively alike. Such was not the case, the growth mass decreased progressively with the decrease in the initial O_2 tension. In the lowest tensions there was lessened multiplication and slower removal of O_2 . The final growth mass in the bottle which originally had 0.5 per cent O_2 , after all the O_2 was consumed, was greatly inferior to that in the tubes which had the initial tensions of 3 or 5 per cent O_2 .

This result appears to be of considerable significance. In the tissues the O_2 tension is low and consequently the multiplication of the tubercle bacillus is slow. Any measure which would tend to lower the tension would decrease still further the rate of multiplication of the organisms, and thus favor recovery of the individual. In tuberculosis, elimination of physical exertion by complete rest in bed and abundant feeding conceivably owe their beneficial results to a lowering of the O_2 tension in the diseased tissue. Complete removal of O_2 would cause the eventual death of the tubercle bacillus.

Although oxygen is essential to aerobic life yet in concentrations higher than that of air, it may be injurious. Lavoisier was the first to point out the deleterious effect of pure oxygen on animals and this fact has been repeatedly confirmed. Of the unicellular organisms, protozoa appear to be particularly susceptible to high oxygen concentrations. Thus, the development of cultures

of *Trypanosoma lewisi* is distinctly inhibited by a concentration of 45 per cent O_2 or about twice that in ordinary air. Cultures of *Leishmania* were found to be less sensitive since they grew perfectly well in 50 per cent O_2 but with 60 per cent oxygen some evidence of inhibition was noted. This was more marked with 80 per cent and was complete with 100 per cent. Certain parasitic and free living protozoa when exposed to pure oxygen under high pressure were found by Cleveland to be actually killed.

Like the protozoa bacteria show a varying response on exposure to high tensions of oxygen. In general however they are considerably more resistant. The glanders bacillus showed no difference in growth or gas exchange when grown in air and in 99 per cent oxygen. The human and bovine strains of the tubercle bacillus were apparently stimulated by tensions of 40 to 60 per cent. Good growth was obtained in 80 and 100 per cent oxygen but it was not evenly distributed over the surface. Instead isolated heaped-up colonies developed. The organisms outside of these colonies were killed. Apparently, the bacilli in the small masses of the inoculated material were protected against the injurious effect and were able to multiply whereas the isolated bacteria were destroyed. It is possible that a perfectly distributed inoculum would be completely inhibited in its growth and even destroyed by exposure to 100 per cent O_2 . All told about six bacteria have been found by others to be inhibited by high concentrations of oxygen but these results are open to the objection that soda-lime was used in the containers. The removal of CO_2 is in itself an inhibiting factor.

Varying CO_2 Concentrations—When an organism is grown in a limited air volume the oxygen is completely consumed and is replaced by 17 to 21 per cent of CO_2 , depending upon the nature of the substance which is being utilized. The respiratory quotient for glucose is 1.0 which means that the volume of CO_2 produced equals that of the O_2 consumed. It follows therefore that bacteria and protozoa are not inhibited by such concentrations of CO_2 .

The effect of increased concentrations of CO_2 is more marked with protozoa than with bacteria. Cultures of *Trypanosoma lewisi* failed to grow in 22 per cent CO_2 . *Leishmania tropica* grew well in 20 per cent CO_2 but failed to develop in 29 per cent. The trypanosome culture is more sensitive than *Leishmania* to increased concentration of O_2 or of CO_2 .

In testing the action of high concentrations of CO_2 it is very desirable that the containers shall each receive about 100 c.c. of O_2 per tube of culture to insure abundant O_2 for development. This necessitates the use of larger jars in tests with high CO_2 concentrations than are needed for those with lower tensions. When sufficient oxygen is provided the tubercle bacillus will grow even in 90 per cent CO_2 . The growth however is slow to develop and is not as rich as in 90 per cent O_2 . In general it may be said that concentrations of CO_2 up to 50 per cent have little or no effect upon the growth of the tubercle and glanders bacilli. At 60 per cent and above the respiratory rate is decreased and as a result the growths are less rich. No difference however is observed in the respiratory quotients.

The effect of low concentrations of CO_2 is of no particular significance for

it is conceivable than an organism may grow in the open air in which case the CO_2 produced would be dissipated about as fast as made and of course the tension of CO_2 in the air (0.04 per cent) would be unaffected. Indeed it is possible to obtain an excellent growth in an atmosphere in which the tension of CO_2 is apparently nil. This fact was brought out in the following test: a glycerine agar plate, inoculated with the bovine tubercle bacillus, was placed in an inverted position, without the cover, in a jar through which CO_2 free air was forced at the rate of 600 cc per minute for twenty-one days. No CO_2 could be detected by buet analysis and yet a very rich culture developed. It would be wrong to conclude from a test such as this that the organism grew in a CO_2 -free atmosphere. As a matter of fact the germ grew in a film of moisture which was not CO_2 -free. The condition parallels growth in a broth culture over alkali (Table XIII).

Complete Removal of CO_2 —A considerably more important question is the growth of organisms in the absence of CO_2 . Wheery and Ervin were the first to show that the tubercle bacillus failed to grow in the presence of alkali. In other words the removal of CO_2 from a culture resulted in inhibition of growth. This observation was confirmed and extended by Rockwell and by Valley and Rettger. The interpretation of their results, however, was open to question, since it seemed that something more was involved than the mere removal of CO_2 from the air and medium. It was conceivable that intracellular CO_2 was an essential in cellular equilibrium, and hence, in the respiratory process. Its complete withdrawal from a cell would therefore alter the equilibrium and as a consequence respiration would cease and death result.

Definite evidence of the presence and amount of intracellular CO_2 in bacteria has been wanting. Accordingly, tests were made with human and bovine tubercle bacilli, with a saprophytic tubercle bacillus and with the hay bacillus. The results obtained with the latter organism are presented in Table XII where it will be seen that in 1.9 gm of the thoroughly dried cells only 0.2 or 0.32 cc of CO_2 was present. The same amount of cells, in the moist washed condition, held 1.72 cc of intracellular CO_2 , the major part of which is lost by desiccation over alkali. Small as is this amount of CO_2 it nevertheless appears to be of great significance in the viability of the cell.

TABLE XII
TOTAL INTRACELLULAR CO_2 IN WET AND DRY *B. SUBTILIS* CC AT 0° C, 760 MM *

EXPERIMENT NUMBER	3	4
CO_2 in air control		0.09
CO_2 in washed cells	1.44 (5)	1.72 (5.3)
CO_2 in dried cells	0.22 (5)	0.20 (5.3)
Weight of dried cells		1.91 g (5.3)
CO_2 present		0.32

*The figures in parenthesis indicate number of Roux flasks used.
Cells dried in N_2 over P_2O_5 and KOH for two and thirteen days respectively.

While under certain conditions growth can be inhibited on the surface of a solid medium such as agar or serum it is not possible to bring about this result with an inoculated broth. This is shown in an experiment the results of which are given in Table XIII. In this test the inoculated broth in an open

TABLE XIII
CULTURE OF *B. SUBTILIS* IN GLYCEROL BROTH OVER ALKALI AND OVER WATER*

TEST NO	KEPT OVER		
	ALKALI		WATER
	1	2	3
Inoculated broth			
Gaseous CO ₂	0	0	1.12
Dissolved CO ₂	1.04	1.32	2.56
Control			
Uninoculated broth			
Gaseous CO ₂	0		0.04
Dissolved CO ₂	0.60		0.88

*The figures represent volume per cent of CO₂

Petri dish was set in a jar. Alkali was placed above and below the culture dish to remove the free gaseous CO₂. Analysis showed the absence of CO₂ from the air of the jar and yet a typical rich growth developed in the broth. Analysis of the broth, however, revealed the presence of 1.04 and 1.32 volume per cent of CO₂. The alkali was able to effect complete removal of CO₂ from the air in the jar but was not able to do this to the broth itself. The organism consequently was exposed to a certain tension of dissolved CO₂ although the overlying air was practically free of the gaseous form. It is evident from this and like tests that in the presence of alkali or streaming CO₂-free air, even a film of water will favor multiplication, presumably by preventing a rapid loss of intracellular CO₂.

There are other factors besides a relatively dry surface of the solid medium which must be taken into account in experiments designed to test the question of the growth of organisms in the absence of CO₂. The nutrient quality of the medium is likewise a determining factor. On the ordinary beef infusion agar it is quite impossible to secure inhibition of rapidly growing organisms by exposure to alkali. A growth once started will be wholly unaffected by the presence of an absorbent. The less nutritive extract agar by prolonging somewhat the lag period favors the result sought. Adjustment of the reaction to the acid side, such as P_H 6.0, is of distinct value. The typhoid bacillus in the presence of alkali, grows unhindered on infusion agar but is completely inhibited on extract agar P_H 6.0.

The use of a broth suspension of the organisms to be tested is undesirable because it furnishes a readily available nutrient medium. Moreover, even if quickly dried, it forms a protective film around the organisms. Hence it is preferable to use as the inoculum suspensions made with distilled water. A drop or two of this water suspension is placed on the dry surface of the medium and spread about by a bent glass rod so as to secure as far as possible perfect distri-

bution This cannot always be accomplished and some piling up of cells in minute masses may occur Such grouping of cells affords mutual protection and as a result colonies may develop on the medium in the presence of alkali

By observing the conditions mentioned it is possible to obtain complete inhibition of growth when the inoculated medium is exposed to the action of an absorbent An alkali, however, is not necessary for the same result can be secured by exposure to a large volume of N_2 , as in an autoclave, or to streaming nitrogen, or to streaming CO_2 -free air, or in a vacuum

Inhibition of growth, however, is not the most significant fact arrived at by these tests If, after a given exposure, the plate is taken out of the apparatus and set aside in the incubator, it will be found that rapid destruction of the organism has taken place

This reduction in viability will be noted in Table XIV In these experiments the hay bacillus was exposed, over alkali, in nitrogen No visible growth developed But when removed and incubated in the air, after an exposure of four hours, the plate showed an appreciable decrease in the number of colonies as compared with the control plate The reduction was very marked at the end of eight hours and after 12 hours was nearly complete

TABLE XIV

REDUCTION IN VIABILITY OF *B. SUBTILIS* BY EXPOSURE OF INOCULATED PLATES IN N_2 OVER ALKALI AT $33^\circ C$ *

OVER ALKALI IN N_2	THEN CHANGED TO OVER WATER IN AIR PLATES GAVE
Hours	
4 (2)	100 colonies each
8 (2)	4 and 7 colonies
12 (3)	0, 2 and 4 colonies
24 (3)	0 (2) and 3 colonies
36 (7)	0 (6) and 2 colonies

*The figures in parenthesis indicate the number of tests
Control plates developed 800 or more colonies

The same result was obtained with plates exposed to streaming nitrogen or to a vacuum without the presence of alkali, as shown in Table XV The same is true when streaming CO_2 -free air is used

TABLE XV

REDUCTION IN VIABILITY OF *B. SUBTILIS* BY EXPOSURE OF INOCULATED PLATES IN NITROGEN OR IN VACUUM AT $33^\circ C$ *

	HOURS	THEN CHANGED TO AIR OVER WATER GAVE
In streaming nitrogen	8 (2) 12 (2) 24 (2)	150 colonies 100 colonies 0, 3 colonies
In continuous vacuum	8 (2) 12 (2) 24 (6)	100 200 colonies 50 70 colonies 0 18 colonies

*The figures in parenthesis indicate number of tests
Control plates developed 800 or more colonies

In considering the effect of moisture, an experiment was cited in which CO_2 free air, streaming at the rate of 600 c c per minute, failed to inhibit the growth of the tubercle bacillus. The same was true for other organisms tested in like manner. It became evident that the effect, if any, of a stream of CO_2 -free air could only be obtained by passing it directly over the inoculated surface. The Petri plates were therefore discarded and instead Kitasato flasks were used. This modification gave at once the expected results.

TABLE XVI
INHIBITION AND DESTRUCTION OF *B. SUBTILIS* IN STREAMING CO_2 FREE AIR, KITASATO FLASKS,
EXTRACT AGAR, pH 6.0, 37°C AEROBIC GROWTH IN SLOW STREAM
PROTECTIVE ACTION OF CO_2 *

RATE OF FLOW CC PER MIN.	EXPOSURE HOURS	CO_2 FREE AIR		CO_2 FREE AIR +3% CO_2 GROWTH
		PRIMARY COLONIES	THEN PLACED IN AIR 35°C 48 HRS	
200	12	0	7	+ rich
200	24	0	0	+ rich
100	24	1	2	
50	24	0	0	
25	48	0	0	
10	48	0	0	
4	48	0	0	
1	24	+ like control	+ like control	

*The control plates had 200 or more colonies.

The colonies which developed during the exposure period are designated as primary.

The total number of colonies present on the plate after incubation in ordinary air indicates the number of survivals.

Table XVI which shows the effect on the hay bacillus is illustrative of the results obtained with a number of other organisms. With the CO_2 -free air streaming at the rate of 200 c c per minute there was complete inhibition and the flask which was thus treated for twelve hours on subsequent incubation gave only 7 colonies, while that which was exposed for twenty-four hours gave none. Practically all of the organisms were therefore killed in less than twelve hours. The presence of only 7 colonies in one case is probably due to the almost unavoidable grouping of cells in the process of spreading. A massing of this kind was responsible, without doubt, for the presence of 1 primary colony in the flask aerated at the rate of 100 c c per minute.

By dividing the current of CO_2 -free air, it is possible to run duplicate tests or, what is better, to introduce into one branch a slow stream of CO_2 . As shown in Table XVI, the restitution of CO_2 to the main stream of CO_2 -free air resulted in a rich growth the same as in a control flask kept in the room. The rate of flow was the same through both flasks. In the one which received CO_2 -free air there was no growth and death resulted. In the other flask through which passed the same air plus 3 per cent CO_2 there was a rich growth.

A striking demonstration of the effect of absence or presence of CO_2 was obtained with the human and bovine tubercle bacilli, which were tested side by side. A ground up suspension in distilled water of these organisms was spread over the previously dried surface of infusion agar pH 7.4 in Kitasato flasks. The rate of flow was 100 c.c. per minute. The flasks through which streamed CO_2 -free air plus the addition of 6 per cent CO_2 , at the end of twenty-eight days showed solid growths even richer than an ordinary control. On the other hand, the flasks through which only CO_2 -free air passed showed no evidence of growth at the end of twenty-eight days. They were then disconnected and set aside in the incubator at 37°C for one hundred twelve days but no sign of growth appeared. Total destruction of the two strains of tubercle bacilli was brought about by the streaming CO_2 -free air.

The high speed of aeration alone was not responsible for the destructive effect. As shown in Table XVI the reduction of the speed to 4 c.c. per minute gave the same result, viz., destruction of the organism. With a speed of 2 or 3 c.c. per minute many primary colonies developed and on subsequent incubation the growth became the same as on the control plate, showing that no destruction took place. With a speed of 1 c.c. per minute the flask developed a growth like that of the control. Incidentally it may be stated that in a similar slow stream of purified CO_2 -free nitrogen the hay and the typhoid bacillus grew as facultative anaerobes.

It is evident therefore that growth can and does take place in a slow stream of CO_2 -free air. This result is to be expected if it is supposed that the observed injurious effects are due to the depletion of intracellular CO_2 . In a rapid stream the organism is losing its reserve of this gas faster than it is being made, whereas in a sufficiently slow stream whatever loss occurs by diffusion through the cell wall is overcompensated by the respiratory process. Hence the normal intracellular equilibrium is maintained and growth results.

It has been held that CO_2 was utilized as a food and that the failure of organisms to grow on plates exposed to alkali was due to the removal of CO_2 from the medium. This view, however, is not supported by experimental facts. Thus, if an uninoculated agar plate is kept over alkali for twenty-four to forty-eight hours it should be rendered free of the supposedly nutrient CO_2 and consequently would become a poor medium for growth. Again, if through an uninoculated Kitasato flask a stream of CO_2 -free air (100 c.c. per minute) is passed for two to three days it likewise should be made unfavorable as a culture medium. But in either case, after the exposure mentioned, if the medium is quickly inoculated and at once subjected to a slow stream of CO_2 -free air (1 to 2 c.c. per minute) growth results the same as on a control.

Clearly, it is not the medium which is affected by exposure to an absorbent or to a rapid stream of CO_2 -free air. The reasonable conclusion to be drawn is that these agents deprive the inoculated organism of an essential constituent, viz., intracellular CO_2 .

It is well known that some organisms, such as the bovine strain of *B. abortus*, do not readily grow in the primary isolation unless some CO_2 is introduced into the culture jar. The explanation of this peculiar behavior, in the light of

these studies is that the organism on removal from the body loses a considerable amount of its intracellular CO_2 and its growth is thereby retarded. This loss is compensated by placing the inoculated tubes under increased CO_2 tension, and as a result good growth takes place.

The fact that a rapid stream of CO_2 -free air or nitrogen, or exposure to an absorbent destroys organisms can perhaps be utilized to distinguish between the living cell and nonliving inanimate matter. For example the question of the nature of the bacteriophage has been much discussed. If it be a living organism it should be killed by exposure to conditions which effect removal of intracellular CO_2 . A nonliving chemical substance an enzyme for example might be expected to withstand such conditions. In the tests which have been made thus far it has not been possible to decrease or destroy the bacteriophage by CO_2 -free air. This fact speaks against the view that the lytic principle is a living organism. Further work however is necessary before a final conclusion can be drawn.

SUMMARY

By means of exact methods it has been possible to determine the extent of gas exchange for a number of bacteria and protozoa. Respiration is an essential characteristic of living matter, and in the case of aerobic organisms it is evidenced by the consumption of oxygen and the elimination of CO_2 . The rate and intensity of this change varies with the composition of the medium and the kind of organism. The respiratory quotients in general approach the theoretical values. The effect of varying concentrations of oxygen and of carbon dioxide are shown. The reduction or removal of intracellular carbon dioxide results in the death of the cell and this fact perhaps may be used in determining the nature of the so called filtrable viruses.

A COMPARATIVE STATISTICAL STUDY OF THE FREQUENCY OF ASYMPTOMATIC RINGWORM AS OCCURRING IN THE MORE COMMON CUTANEOUS AFFECTIONS*

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THIS investigation has a threefold purpose an evaluation of the disputed microscopic forms of ringworm hyphomycetes, demonstration of some possible relationship between asymptomatic ringworm infection and certain of the common dermatoses, and lending further support to the potential possibilities of the silent (asymptomatic) form of mycotic infections of the feet as a causative factor in the production of cutaneous disease. The lack of appreciation of the importance of this problem probably rests upon the skepticism concerning the pathogenicity of some of the forms of ringworm fungi found in the silent type doubt as to the true nature of the "mosaic" type of ringworm hyphomycetes, belittlement of the comparatively insignificant lesions of asymptomatic epidermophytosis, and failure of realization that the lesions of the silent type may assume active (symptomatic) form.

Both the clinician and the laboratory worker have attempted to define definitely the status of this problem. The clinician, with full realization of the frequency with which fungi (the ringworm hyphomycetes) are etiologically responsible for vesicular and squamous eruptions of the hands and feet, has not failed to take into consideration the causative possibilities of the various occupations ("Occupational dyshidrosiform dermatitis of Darier"), as well as the influence of disfunctions of the sympathetic nervous system in the production of eruptions closely simulating those caused by the ringworm hyphomycetes. The laboratory has shown that yeasts and even cocci may bear an etiologic relationship to cutaneous lesions similar to those produced by ringworm fungi.

As the "mosaic" spores are frequently encountered in microscopic preparations of epidermal tissues of asymptomatic epidermophytosis, their exact status becomes a matter of greatest importance. Becker and Ritchie believe that the "mosaic" type represents a collection of organic material which may result from inflammatory changes in the tissues. These authors were able to produce forms simulating the "mosaic" configurations through admixture of potassium hydroxide and olive oil, but the double contour was lacking. F. Weidman concludes that he does not believe all of the mosaic forms to be true forms of fungus. Greenwood and Rockwood, however, are convinced of "the identity of the somewhat bizarre, degenerate 'mosaic' form with the usual actively growing mycelium." They were able to demonstrate the continuity of these forms with normal mycelium in the same scale.

G. M. MacKee and G. M. Lewis in a recent contribution entitled, "Keratolysis Exfoliativa and the Mosaic Fungus," consider the question of the

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"mosaic" fungi at great length and the following are the most important of their conclusions

1 The "mosaic" fungus is frequently demonstrated in various types of dermatomycoses in association with accepted forms of pathogenic fungi

2 "Mosaic" fungi are rarely demonstrated in diseases other than the conventional dermatomycoses or dermatophytid of the hands and feet

3 Pathogenic growths have been cultivated in a few instances from the scales and vesicular tops of ringworm lesions when only "mosaic" forms could be demonstrated in extemporaneous preparations

4 Artificially produced blisters gave negative results for fungus, excepting in locations usually predisposed to dermatomycoses with the patients giving

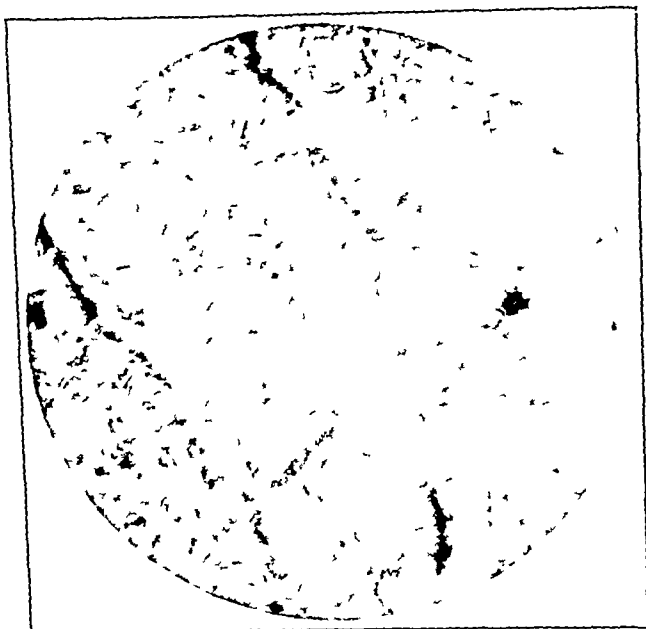


Fig 1—Mycelia found in epidermal tissue of vesicle before treatment January 21 1931

either the history of a previous attack of dermatomycosis or of a fungus focus located either on the hands, feet, or between the toes

5 Use of xylene preparations and the absence of the "mosaic" forms in the fatty scales of seborrhea seem to disprove that these forms are artifacts the result of chemical union of fat globules with potash solutions

6 If a case of dermatomycosis is examined repeatedly for a long while, the conventional hyphae may be found at one time the "mosaic" form at another, or both may be found in the same specimen

In this connection the following clinical and laboratory observations of one of us (A S) are worthy of record

M G consulted (A S) for a skin condition involving the palms and fingers on both hands, duration nine months The skin was thickened fissured, scaly, and presented a picture considered typical of eczema squamosum Repeated examination of the scales removed from the hand lesions proved negative for fungi

The feet showed but little pathology, excepting for slight scaling of the plantar surface of the left big toe. Repeated examination of the epidermal scrapings of the feet lesions showed only "mosaic" spores. After three weeks' treatment the affected skin surface of the feet appeared clinically normal, and microscopic examination of the removed material showed absence of fungus. The active lesions of the hands showed marked improvement under treatment. About five weeks after beginning treatment, the condition of the hands seemed to remain stationary and almost coincidental with this, the patient complained of an eruption on the plantar aspect of the left big toe. Examination disclosed a few vesicles in this area together with rather marked scaling. Microscopic study of the vesicular type showed the presence of typical mycelia.

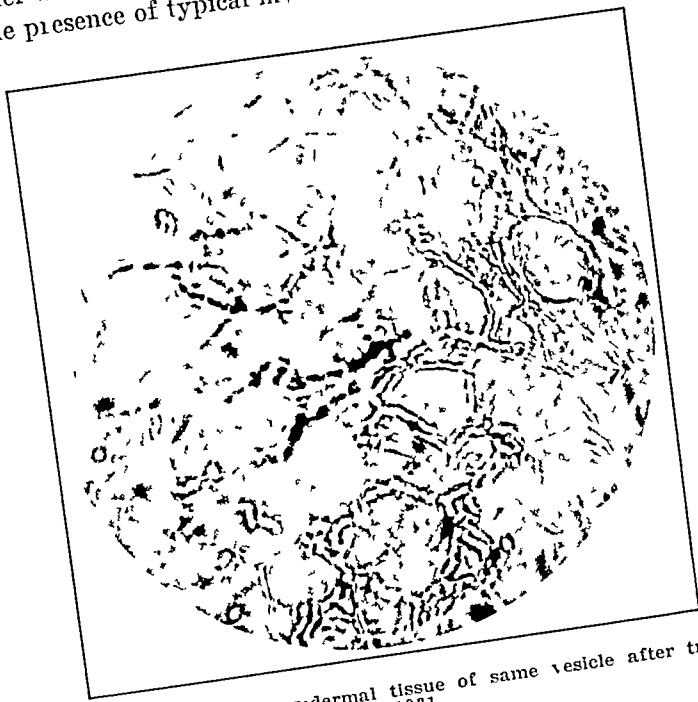


Fig 2 — Mosaic spores found in epidermal tissue of same vesicle after treatment January 24 1931

A W presented as part of his cutaneous eruption a few typical steel colored vesicles on the inner aspect of the right big toe. Opportunity was offered (A S) to observe these lesions daily and to remove material for study. The following photographs show the mycelia of the active stage as well as the "mosaic" spores of the rather unfavorable environment created through treatment.

The evidence cited here, together with the observations of Greenwood and Rockwood, and MacKee and Lewis form to our minds uncontradictory evidence that "mosaic" spores are a true form of ringworm hypomyces.

In addition to the consideration of the status of the "mosaic" spores one of the objectives of this investigation was the determination of the pathogenicity of the fungi of the silent form of feet mycosis. Only one of Koch's postulates was studied, i.e., ability to grow the causative organism in artificial culture media. Epidermal scrapings from each of the 185 patients studied were cultured in

Sabouraud's media according to the usual technic Dr Ralph P Zalatel, Pathologist of The Skin and Cancer Hospital of Philadelphia was responsible for the cultural findings

Analysis of the data obtained in this investigation justify the following statements

One hundred and eighty-five patients were examined, and out of this number, 92, or a trifle over 49 per cent were positive for fungi on extemporaneous examination while 93 or a portion over 50 per cent were negative Only in 4 instances, or in a little over 2 per cent was it possible to obtain a typical ringworm growth on Sabouraud's media

The last phase of this investigation, an attempt to associate asymptomatic epidermophytosis with one or another of the more common dermatoses is here presented with the following results

Forty-five patients with acne vulgaris were examined for ringworm fungus infection of the feet Their ages ranged from thirteen to twenty-nine years Of this number, 27 or 60 per cent showed negative microscopic findings for fungi while 18, or 40 per cent proved positive Epidermal material from the interspaces of all of the 45 patients were cultured on Sabouraud's media and in only one instance was a typical ringworm fungus growth obtained Scaliness of the interspaces was present in all cases, except in one instance where the interspaces appeared to be apparently normal Exfoliation was an additional feature in 28 out of 45 patients studied In the 27 negative fungus cases, 14 showed involvement of one to three interspaces and 13 cases showed involvement of all the interspaces Of the 18 positive cases, 9 showed involvement of one to three interspaces and 9 showed involvement of all interspaces

Fourteen patients with eczema were studied, 5 of the vesicular variety, 6 of the papulo-squamous type and in 3 instances the type was not given Of this number, 3 or 22 per cent were found positive on microscopic fungus examination and 11 or 78 per cent were negative All failed to give a typical ringworm growth on Sabouraud's media Out of the 14 patients, 7 showed involvement of all the interspaces and 7, one to three interspaces Scaliness was present in all of the patients with eczema Exfoliation was found in only 7 instances

Ten patients with seborrheic dermatitis were studied as follows

Five or 50 per cent were positive for fungi on extemporaneous examination and 5 or 50 per cent negative Specimens of all the 10 cases cultured on Sabouraud's media failed to give a positive result In 6 instances all the interspaces were involved and in 4, only several All cases showed scaliness of the interspaces

The miscellaneous group included the following 29 patients with dermatitis, 5 with seborrhea, 5 with psoriasis 6 with herpes, 4 with urticaria, 4 with rosacea, 4 with scabies, 10 with various cutaneous pus affections, 5 with varicose ulcers, 4 with pruritus, 4 with alopecia, 3 with tertiary syphilis, 6 with various types of dystrophy of the skin, 3 with miliaria, 3 with lichen planus, and 15 with benign and malignant skin tumors

This series constituted 110 patients and of this number 51 or 46 4 per cent were microscopically negative for ringworm fungi and 57 or 51 8 per cent posi-

tive Only two of this group (1.8 per cent) gave positive cultures on Sabouraud's media

Individual statistics of the more prominent cutaneous affections included in the above are shown in Table I

TABLE I

NAME	NO OF PATIENTS	PER CENT OF POSITIVE MICROSCOPIC FUNGUS FINDINGS	PER CENT OF NEGATIVE MICROSCOPIC FUNGUS FINDINGS
*Dermatitis	29	55.2	44.8
Seborrhea	5	40	60
Psoriasis and Lichen Planus	8	12.5	87.5
Scabies	4	50	50
Varicose Ulcers	5	20	80
Tumors	17	53.33	46.66
Pus affections	10	60	40

*In one case of dermatitis a positive culture on Sabouraud's media was obtained

RÉSUMÉ

1 One hundred and eighty-five patients suffering from various skin affections were examined for the presence of ringworm fungi in the interspaces of the feet, both by the extemporaneous method and by culture on Sabouraud's media

2 Of this number, 92 or 49 per cent were proved positive for fungi on microscopic examination and 93 or 51 per cent negative

3 Only in 4 instances or 2 per cent could a positive ringworm growth be obtained on Sabouraud's media

4 Forty-five patients with acne vulgaris were studied Of this number, 27 or 60 per cent were negative for fungi by the extemporaneous method, while 18 or 40 per cent were positive Out of the 45, there was only one case which gave a positive ringworm growth on Sabouraud's media

5 Fourteen patients with eczema were studied with the following results Three or 22 per cent were positive for fungi by the microscopic method and 11 or 78 per cent were negative All failed to give a ringworm growth on Sabouraud's media

Eight patients with either psoriasis or lichen planus were studied, and of this number 12.5 per cent showed positive microscopic findings and 87.5 negative results

6 Ten patients with seborrheic dermatitis were examined Of this number 5 or 50 per cent were positive for fungi by microscopic examination and 5 or 50 per cent negative All failed to yield positive cultures on Sabouraud's media

7 Patients with various forms of dermatitis, scabies or tumors showed about an equal proportion of positive and negative microscopic findings

8 While the general average of positive fungus findings by microscopic examination was 49 per cent for the entire series, the eczema group only yielded 20 per cent positive results, acne vulgaris 40 per cent, but the seborrheic dermatitis group gave 50 per cent positive Although this series is rather small upon which to base definite conclusions, it would appear that the eczema patients seem

to offer the least favorable soil for asymptomatic epidermophytosis and seborrheic dermatitis patients the most favorable environment

9 Statistical study of the frequency of asymptomatic mycosis of the feet is well recognized. However, doubt as to the pathogenicity of the ringworm fungi encountered in this affection still remains. This investigation is an attempt to show that these fungi through their ability to grow in favorable culture media outside the body probably possess potential powers of assuming the same rôle in the human skin when conditions become favorable. Since it was found impractical to fulfill all of Koch's postulates particularly reinfection of human beings with ringworm fungi this work was not carried on beyond the attempt to culture on Sabouraud's media.

10 An important lesson to be drawn from this paper is the need for a test, cutaneous or otherwise, through which the potential, latent pathogenic powers of the fungi of asymptomatic ringworm fungus infection could be determined. By such a method it would also be possible to reconcile the discrepancies between the cultural and microscopic findings of asymptomatic mycosis.

CARBOHYDRATE IN THE TREATMENT OF POSTOPERATIVE TETANY, WITH SPECIAL REFERENCE TO LACTOSE*

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THE usual methods of treatment of postoperative tetany are not entirely satisfactory. To any who have experienced the difficulties in the management of this condition, the need of simpler effective measures is apparent. The oral administration of calcium, even in large doses, is not always sufficient to control the symptoms, and repeated injections of it over long periods is undesirable or impossible. Injections of parathyroid extract alone or in addition to calcium may be effectual, but are inconvenient and expensive. Methods are reported here for the control of phosphate metabolism to an extent that will afford distinct benefit to patients suffering from this disease.

SERUM CALCIUM

The best-known and perhaps the most satisfactory single criterion by which the severity of parathyroid tetany may be judged is the degree of depression of the level of total serum calcium. Examination of a large series of serum calcium levels in tetany makes the fact apparent, however, that the symptoms do not necessarily parallel the total calcium levels. This confirms the opinion of John¹

It is known that the total serum calcium can be raised and frequently brought to normal in tetany by the feeding of large doses of calcium. In some of our cases the symptoms were not controlled by these measures, and their severity was thought to be out of proportion to the calcium level. This was especially striking since a patient might be symptom-free with a certain calcium level on one day, while on another occasion, although the serum calcium was at the same height, symptoms might be present. Abnormally high levels of blood phosphates accompanied nearly normal calcium values in some of these cases.

BLOOD INORGANIC PHOSPHATES

Ver Eecke² in 1898 noted a lessened phosphate excretion in the urine in tetany. This has received ample confirmation by Salvasen,³ Greenwald,⁴ and others, and it is now recognized that one of the constant features of parathyroid tetany is phosphate retention. Furthermore, tetany has been produced experimentally by feeding phosphates.⁵ The phosphate retention in tetany usually is associated with a distinct rise of blood phosphates, the concentration of which partially governs the severity of the symptoms. This is definitely indicated by the observations reported here and by a review of the literature. Pronounced mitigation of symptoms accompanying a fall in blood phosphates has been ob-

*From the Cleveland Clinic.
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served and simultaneously the neuromuscular electrical excitability approaches normal

Calcium phosphate is a relatively insoluble compound. It has been stated that the blood is supersaturated with this salt.^{7, 8} This has been questioned,⁹ but it is certain that it approaches the saturation level. Under these conditions, it might be expected that a decrease in blood phosphates would result in an increase in serum calcium.⁹ It has been observed more frequently in these studies, however, that there is a slight fall in total serum calcium accompanying the fall in blood phosphates. It is possible that the relief of symptoms associated with this fall in phosphates is the result of an increase in the percentage of calcium ionized. It is also possible that the decrease in phosphates independently decreases neuromuscular excitability.

RELATION OF CARBOHYDRATES TO BLOOD PHOSPHATES

At the beginning of this century it was generally accepted that phosphate metabolism was not normal in diabetes mellitus.^{10, 11, 12} Since that time intensive study has demonstrated an extraordinarily close relationship between carbohydrate and phosphate metabolism. Harrop and Benedict¹³ showed that in normal glucose-tolerance curves, the level of blood phosphate fell, the low point of the phosphate curve being subsequent to the highest glucose level. They believed that phosphates are utilized temporarily during the transference of glucose from the blood. This has been corroborated by many writers.^{14, 15, 16}

It has been shown that in dogs suffering from tetany, the symptoms are more pronounced on a meat than on a carbohydrate diet. Dragstedt¹⁷ states that dogs with tetany lived longer when fed milk, white bread, and lactose than the usual survival period for thyroparathyroidectomized dogs. In spite of insufficient evidence, his conclusions appear to be correct. Blood studies were not made in his series, and the improvement was considered to be the result of changes in the gut. That the Dragstedt diet is beneficial in tetany has received confirmation. Inouye¹⁸ and Frank, Haring, and Kuhnau¹⁹ also contend that the beneficial effect of lactose is due to changes in the intestinal tract since parenteral administration is not effective. This conclusion is not completely justifiable, for lactose is not absorbed as such but is first hydrolyzed, with the formation of glucose and galactose. Hydrolysis takes place only to a small extent however, if the lactose is given intravenously.²⁰

Dragstedt believed that benefit was obtained in tetany by a diet of white bread, milk, and lactose because this diet prevented the absorption of toxic substances. Salvasen³ criticizes Dragstedt's work, and in an excellent treatise presents evidence to prove that the entire benefit of milk is produced by its calcium content. He makes little comment on the effect of milk on blood phosphates. Although there is no question that calcium is essential, the studies herein reported make it apparent that the efficacy of this diet is partly attributable to its effect on phosphate metabolism.

In studying carbohydrate metabolism in parathyroidectomized dogs, Reed²¹ found that not only ingestion but also injection of dextrose tends to alleviate symptoms of tetany, causing a decrease in inorganic phosphates and a less pronounced decrease in calcium.

THE OBJECT OF THE STUDY

From the preceding statements it is apparent that an increase in the level of inorganic phosphates in the blood may be closely associated with the production of symptoms in tetany. A fall in inorganic phosphates may cause alleviation of symptoms, even though it is not accompanied by a rise in serum calcium.

The object of this study was to find therapeutic measures which would lower the abnormally high level of blood phosphates, with the expectation that clinical improvement of the patient would result. Since there is such an intimate relationship between carbohydrate and phosphate metabolism, it seemed possible that the level of blood phosphates in tetany might be governed by the proper regulation of carbohydrate assimilation.

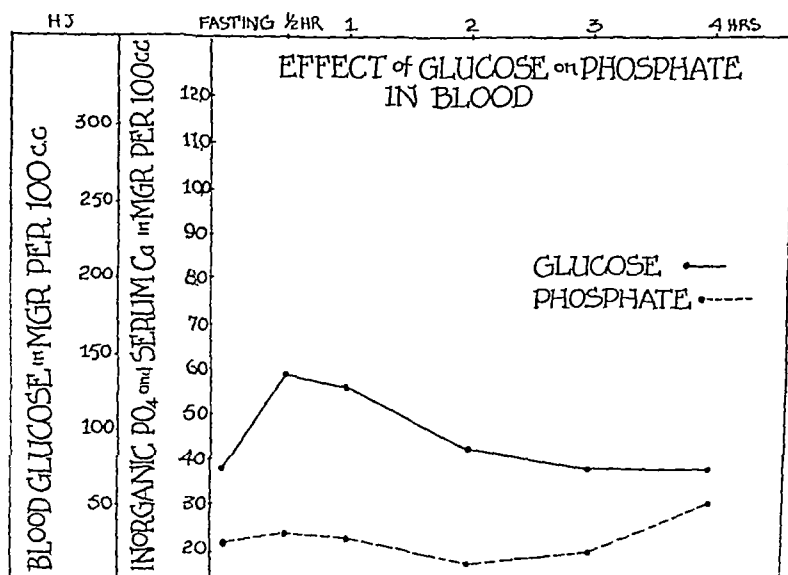


Chart 1

TABLE I

EFFECT OF GLUCOSE ON NORMAL INDIVIDUALS

TIME IN HOURS	FASTING	1/2	1	2	3	4
Case 224731						
Sugar, mg per 100 c.c whole blood	83	155	154	119	52	75
Phosphate, mg per 100 c.c whole blood	3.22	2.77	3.09	2.40	2.21	3.25
Case K C						
Sugar, mg per 100 c.c whole blood	79	86	111	63	54	53
Phosphate mg per 100 c.c whole blood	3.64	3.07	3.31	2.58	2.31	2.78
Case L H						
Sugar, mg per 100 c.c whole blood	99	113	115	88	90	90
Phosphate, mg per 100 c.c whole blood	3.28	2.23	2.57	2.25	2.34	2.50

EFFECT OF GLUCOSE ON BLOOD PHOSPHATES IN NORMAL INDIVIDUALS

In Table I and Chart 1 are given results which confirm the finding that inorganic phosphate disappears from the blood after the administration of glucose in normal individuals

The technic employed is as follows. A specimen of venous blood was obtained after the patient had been without food for twelve to fourteen hours. One hundred grams of glucose were fed, and samples of venous blood were obtained at intervals of half an hour and one, two, three, and four hours, respectively, after the administration of the glucose. Blood sugar and phosphate estimations were made on each sample of blood. Blood sugar was estimated by the method of Hagedorn and Jensen²². Phosphates were measured by the colorimetric procedure of Kuttner and Cohen²³ (Table I, Chart 1).

An examination of these results demonstrates that the lowest point on the phosphate curve usually appears later than the highest point of the sugar curve. Not infrequently the phosphates return to the normal fasting level before the end of four hours.

EFFECT OF GLUCOSE ON BLOOD PHOSPHATE, SERUM CALCIUM, AND NEUROMUSCULAR EXCITABILITY IN CHRONIC PARATHYROID TETANY

The method used was the same as that described above, with the following additions:

Serum calcium determinations according to the Clark and Collip²⁴ modification of the Kramer and Tisdall method were made on the fasting specimen and on two of the other specimens. The electrical neuromuscular excitability (Erb's sign) was measured immediately before obtaining each specimen of blood. The procedure was as follows:

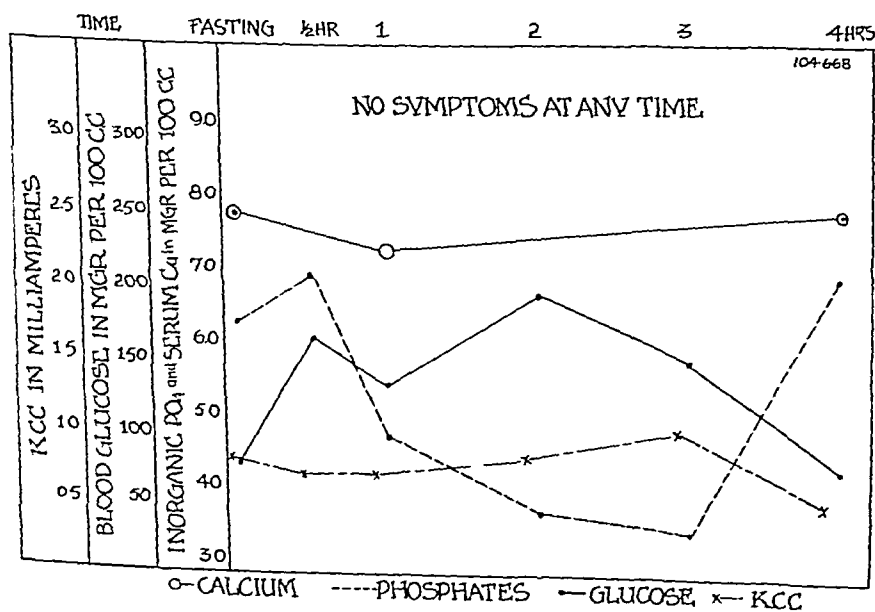


Chart 2

One of the patient's hands was placed on a large moist electrode connected to the anode of a circuit through which a variable direct current could be passed. The cathode consisted of a small metallic terminal covered with wet chamois. The skin over the median nerve at the wrist on the opposite side was moistened, and the electrode was applied. The circuit would be closed or opened by means of a small switch near the cathode. The amount of current running through the circuit was read from a milliammeter. In each case the current was gradually increased until a point was reached when, on closing the switch leading to the cathode, a contraction could be noted in the hand. The number of milliamperes necessary to produce the contraction was called the cathode closing contraction (KCC). A decrease in this figure represents an increased irritability.

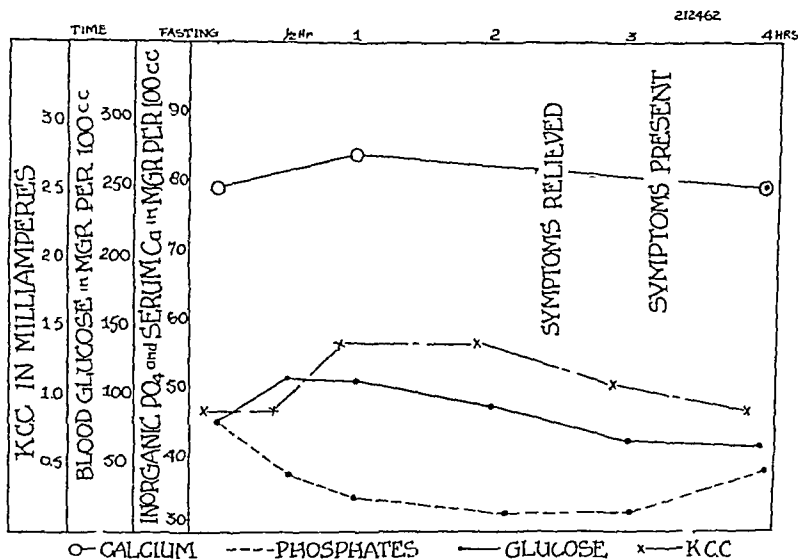


Chart 3

The patients on whom the following studies were made developed chronic parathyroid tetany following thyroidectomy. In each case Trousseau's, Erb's and Chvostek's signs were present, the latter only at times. Abnormally low blood calcium and high blood phosphate levels were always found as shown in Table II. (Two cases which are not included in the tables are graphically depicted in Charts 2 and 3.) The blood sugar curves were essentially normal. The blood calcium changes were small and not constant. In four cases there was an increase, followed by a decrease. In two, there was a decrease which persisted until after the end of the experiment. In one case a decrease was followed by an increase, and in one there was no change. The phosphates invariably were decreased. Only in Case 3 did the decrease continue for as much as four hours. This was associated with a more prolonged rise in the blood sugar than in any of the other cases. The neuromuscular excitability showed an almost constant tendency to decrease during the test. Only in Case 5 was there a definite increase in excitability. In all cases in which there were symptoms at the beginning of the test the symptoms were definitely improved during the test, but in-

TABLE II
EFFECTS OF GLUCOSE ON CHRONIC TETANY

TIME IN HOURS	SUGAR MG PER 100 CC WHOLE BLOOD	CALCIUM MG PER 100 CC SERUM	PHOSPHATE MG PER 100 CC WHOLE BLOOD	CATHODE CLOS- ING CONTRAC- TION	SYMPTOMS
Case 1, No 222908					
Fasting	71	4.8	5.21	1.6	Present
1	133	—	4.74	1.6	Present
1	161	5.8	4.74	1.8	Improved
2	126	—	4.21	1.9	Improved
3	61	—	4.16	1.6	Present
4	59	4.8	4.87	1.4	Present
Case 2 No 124350					
Fasting	65	6.8	6.50	0.8	Present
1	120	—	4.05	0.8	Present
1	87	6.8	5.30	0.7	Improved
2	87	—	3.60	0.8	Improved
3	58	—	3.00	0.7	Present
4	52	6.8	4.70	0.6	Present
Case 3 No 222469					
Fasting	82	7.7	4.07	1.7	No definite symptoms
1	142	—	3.30	1.9	
1	182	7.3	3.66	1.9	
2	158	—	2.88	1.8	
3	117	—	2.42	1.8	
4	76	7.3	2.42	1.7	
Case 4, No 220617					
Fasting	79	8.2	4.70	0.7	Present
1	154	—	4.70	1.2	Present
1	134	9.2	4.00	0.8	Improved
2	109	—	4.10	0.7	Improved
3	70	—	4.40	0.7	Present
4	54	8.2	4.10	0.7	Present
Case 5, No 219959					
Fasting	83	7.8	5.50	1.6	Present
1	155	—	5.70	1.4	Symptoms better
1	129	7.3	4.80	1.1	
2	111	—	3.30	0.9	Present
3	68	—	3.45	1.1	
4	71	7.3	3.48	1.4	Present
Case 6, No 109391					
Fasting	67	6.3	4.80	0.6	Present
1	81	—	5.60	0.7	Gradual improvement
1	98	6.8	4.00	—	
2	89	—	3.20	0.6	
3	70	—	3.75	0.5	
4	70	6.3	3.75	0.4	Present
Case 7 (Control, no glucose given) No 223213					
Fasting	73	7.3	6.82	0.9	
1	75	—	6.40	1.0	
1	79	7.3	7.50	1.0	
2	77	—	6.40	0.6	
3	72	—	6.82	0.7	
4	70	7.3	6.40	0.6	

creased in severity at the end of the experiment when the phosphate level rose. Apparently the symptoms paralleled the phosphate curve more than that of the serum calcium.

TABLE III

EFFECT OF LACTOSE AND GLUCOSE IN NORMAL DOGS

Dog No 1, Weight 38, Experiment No 1, Effect of Glucose, Dose 15 Grams

TIME IN HOURS	FASTING	$\frac{1}{2}$	1	2	3	4	5
Sugar mg per 100 cc whole blood	92	101	132	95	92	84	—
Phosphate mg per 100 cc whole blood	2.39	2.27	—	2.12	2.32	2.39	—
Calcium mg per 100 cc whole blood	11	—	—	—	—	10	—

Dog No 1, Weight 38, Experiment No 2, Effect of Lactose, Dose 15 Grams

TIME IN HOURS	FASTING	$\frac{1}{2}$	1	2	3	4	5
Sugar mg per 100 cc whole blood	77	74	117	75	66	75	—
Phosphate mg per 100 cc whole blood	3.11	2.80	2.87	2.96	3.35	3.30	—
Calcium mg per 100 cc whole blood	12.0	—	—	12.1	—	—	—

Dog No 2, Weight 55, Experiment No 1, Effect of Glucose, Dose 20 Grams

TIME IN HOURS	FASTING	$\frac{1}{2}$	1	2	3	4	5
Sugar mg per 100 cc whole blood	77	90	119	77	74	72	—
Phosphate mg per 100 cc whole blood	2.82	2.06	2.34	2.61	3.41	3.33	—
Calcium mg per 100 cc whole blood	—	—	—	—	—	—	—

Dog No 2, Weight 55, Experiment No 2, Effect of Lactose, Dose 20 Grams

TIME IN HOURS	FASTING	$\frac{1}{2}$	1	2	3	4	5
Sugar mg per 100 cc whole blood	79	79	79	75	81	81	79
Phosphate mg per 100 cc whole blood	3.30	2.77	2.81	2.69	2.84	3.03	3.27
Calcium mg per 100 cc whole blood	8.7	—	8.4	—	8.8	—	—

In Case 7 no glucose was administered. The results were included to show that the changes recorded in the other tables were caused by the glucose.

EFFECT OF LACTOSE ON BLOOD CALCIUM AND PHOSPHATE IN NORMAL DOGS

In one of the following sections it is shown that lactose is of great benefit in the treatment of tetany. The effect of feeding lactose to healthy dogs has been compared to the effect of feeding glucose to the same animals. The methods used were the same as those employed for glucose-tolerance tests. The results are shown in Table III. In this table, the blood sugar is expressed in terms of milligrams of glucose. The curve is very different after lactose feeding from that exhibited after glucose feeding. The glucose curves are similar to those in

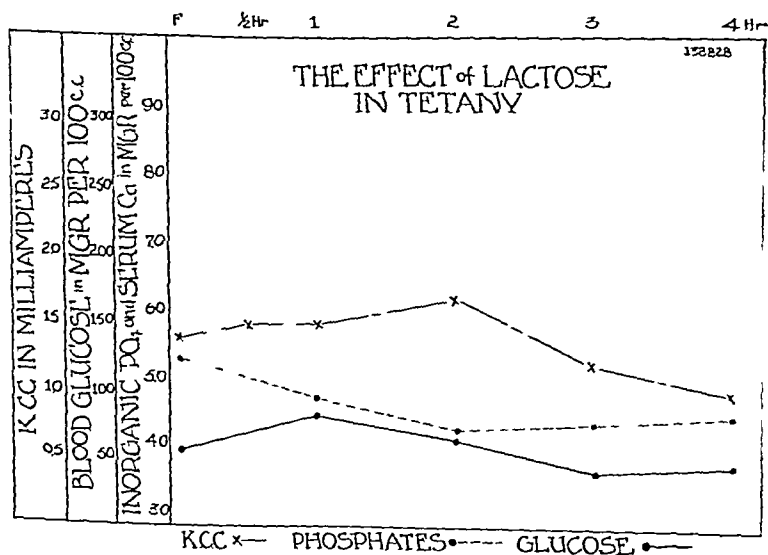


Chart 4

normal individuals, the lactose curves are very low, and indicate either a very high tolerance or very poor absorption. The serum calcium does not show a marked or regular change. There is no difference between the type of phosphate curve obtained with the two sugars, and no indication that lactose produces any prolonged depression of inorganic phosphate in the blood.

EFFECT OF LACTOSE IN CHRONIC TETANY

One hundred grams of lactose were administered to each of four patients suffering from chronic parathyroid tetany. The results are presented in Table IV and Charts 4 and 5. The blood glucose curves are of the same general type as those secured after the administration of glucose to normal individuals. The sugar values tend to be low. Again there is no definite effect on the serum calcium. The depression of inorganic phosphate is about the same as after glucose administration. It appears probable that the lactose is readily digested with the formation of glucose and galactose, and that absorption takes place rapidly.

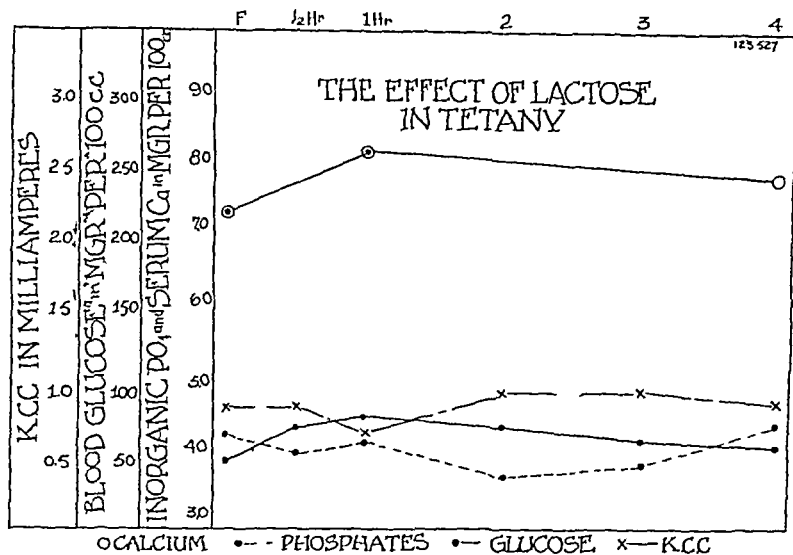


Chart 5

TABLE IV
EFFECT OF LACTOSE IN CHRONIC TETANY

TIME IN HOURS	SUGAR MG PER 100 CC WHOLE BLOOD	CALCIUM MG PER 100 CC SERUM	PHOSPHATE MG PER 100 CC WHOLE BLOOD	CATHODE CLOSING CONTRACTION	SYMPTOMS
Case 8					
Fasting	77	77	5 00	0 7	Present
1	120	—	4 16	0 9	Present
1	117	72	4 16	0 7	Present
2	92	—	3 95	0 8	Improved
3	70	—	3 75	0 8	Improved
4	66	72	3 60	0 8	Improved
Case 9					
Fasting	65		3 85	1 0	Present
1	101		2 90	1 5	Present
1	77		3 56	1 4	Improved
2	77		3 56	1 8	Improved
3	48		3 82	1 1	Present
4	61		3 74	1 0	Present

EFFECT OF GALACTOSE ON BLOOD CALCIUM AND PHOSPHATE IN A NORMAL INDIVIDUAL

Since the glucose resulting from lactose digestion could account for all the changes in inorganic metabolism after lactose feeding, the effect of the galactose moiety was studied separately. The method was the same except that fifty grams of galactose were administered orally. It was considered advisable to use this smaller dose of galactose because of the low tolerance shown by most individuals to this sugar. In the following cases, sugar was excreted in the urine. The results are shown in Table V and Chart 6. As a control experiment, 100 grams of glucose were administered to the same individual (Chart 7). The effect of galactose on blood phosphate is in sharp contrast to that of glucose. This is in

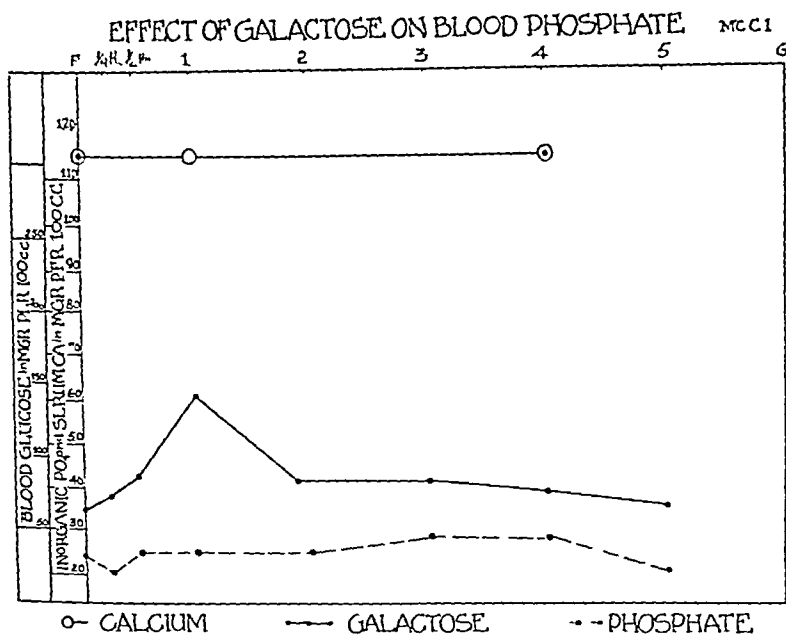


Chart 6

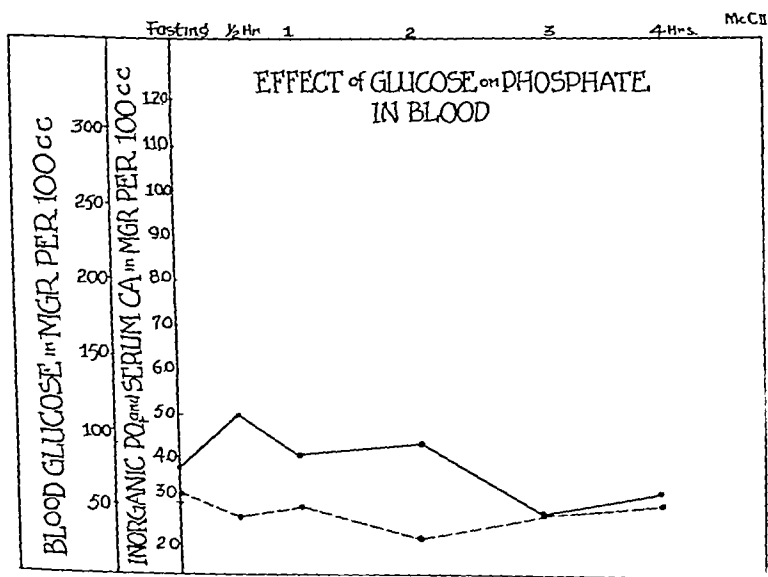


Chart 7

accord with the results of Barrenscheen Galactose does not produce a depression of the phosphate level in the blood

EFFECT OF GALACTOSE IN CHRONIC TETANY

Galactose was administered also to two patients suffering from chronic parathyroid tetany, with the result summarized in Table V. Evidently there is no difference between the reaction to galactose of normal individuals and of those

TABLE V
EFFECT OF GALACTOSE IN CHRONIC TETANY

TIME IN HOURS	SUGAR MG PER 100 C C WHOLE BLOOD	CALCIUM MG PER 100 C C SERUM	PHOSPHATE MG PER 100 C C WHOLE BLOOD	CATHODE CLOSING CONTRACTION	SYMPTOMS
Case No 222908					
Fasting	83	72	376	15	No definite change in symptoms
1	95	—	366	18	
1	104	62	350	18	
2	97	—	368	17	
3	90	—	368	18	
4	83	76	381	18	

Case No 167763

Fasting	65	78	445	09	No definite change in symptoms
1	117	—	366	09	
1	95	79	355	10	
2	102	—	357	10	
3	83	—	364	09	
4	75	73	361	10	

with chronic tetany. No constant changes in inorganic metabolism appear following the administration of this sugar, the neuromuscular irritability does not show changes corresponding to those which occur after the administration of glucose, and the symptoms are not relieved during the test. Thus it seems improbable that it is the galactose moiety of the lactose molecule which results in depression of the blood phosphate. Further studies concerning the mechanism of the action of lactose on blood phosphate are in progress.

TABLE VI
EFFECT OF GLUCOSE, LACTOSE, AND GALACTOSE ON URINE PHOSPHATE EXCRETION

(a) In normal individuals

CASE NO	DOSE	1 HOUR	2 HOURS	3 HOURS	4 HOURS
L H	Glucose, 100 gm	43.8	38.8	29.8	10.5
K C	Glucose, 100 gm	65.9	66.2	58.3	39.6
172516	Glucose, 100 gm	33.3	12.3	0.78	1.05
225005	Glucose, 100 gm	32.1	1.16	1.65	0.95
McC	Galactose, 50 gm	33.2	12.9	27.3	21.0

(b) In chronic tetany

138828	Lactose, 100 gm	42.8	45.1	5.9	3.0
219941	Lactose, 100 gm	21.3	5.2	20.2	1.5
177579	Lactose, 100 gm	14.7	20.8	10.8	3.9
123527	Lactose, 100 gm	22.1	17.1	4.9	13.8
222908	Galactose, 50 gm	—	37.7	15.9	28.7
167763	Galactose, 50 gm	5.2	5.7	4.0	5.4

EFFECT OF GLUCOSE, LACTOSE, AND GALACTOSE ON URINE PHOSPHATE EXCRETION

The fate of the inorganic phosphate which disappears from the blood stream has been considered, and it has been demonstrated that this phosphate is not excreted in the urine. In fact when the blood phosphate is depressed after glucose or lactose administration, the phosphate excretion in the urine diminishes. After the administration of galactose, no definite change develops in the rate of phosphate excretion. This is to be expected, since galactose does not affect the phosphates in the blood stream. There is no difference between the reaction of normal individuals and of those with chronic tetany. The results are given in Table VI. Since the urine specimens were not taken with a catheter, the results given in this table are only approximations. The changes however, are very marked and regular. It seems probable, from this work, that the phosphates which disappear from the blood are carried into the tissues.

LACTOSE IN THE TREATMENT OF CHRONIC PARATHYROID TETANY

In the following cases all calcium and phosphate estimations were made from samples of blood taken after the patient had fasted for approximately twelve hours.

CASE 1—(109391) A young woman eighteen years of age underwent thyroidectomy for adenoma of the thyroid in January, 1921. Two months after the operation she complained of paresthesia and stiffness of the fingers in attacks lasting from a few minutes to a few days. These symptoms persisted. She was treated by oral administration of calcium lactate at irregular intervals. On February 2, 1929, she complained of having had two severe tetanic convulsions. Examination showed the presence of Chvostek's and Trousseau's signs, and the serum calcium was 5.8 mg per 100 cc. From this date, 100 grains of calcium lactate were given daily. The symptoms were partially relieved, but mild symptoms continued.

Chart 8 shows that the average serum calcium level, taken at monthly intervals for a period of nine months previous to November 14, 1929, was 6.6 mg per 100 cc. On Novem

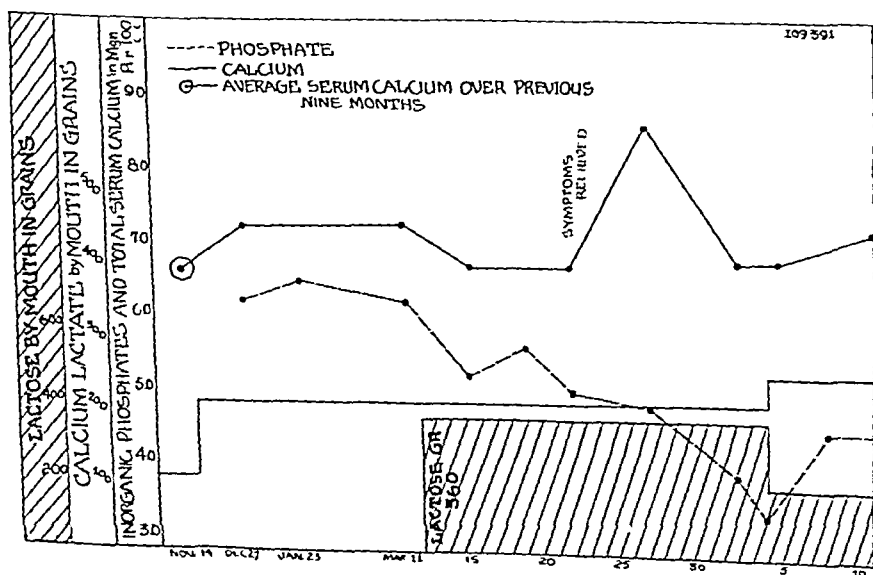


Chart 8

ber 14 the daily dose of calcium lactate was increased to 200 grains. The symptoms were improved slightly, and the serum calcium had risen to 7.2 mg per 100 cc on December 27. On this date the inorganic phosphates were estimated for the first time, and were found to amount to 6.2 mg per 100 cc.

On January 23 the patient was placed on a high carbohydrate diet containing no meat, eggs, or cheese. In the hope of lowering the phosphate level, extra nourishment, consisting of candy, cake, biscuits, or fruit juices, was advised between meals and at bedtime. Between January 23 and March 11, she had five severe attacks of tetany, the symptoms always being more noticeable in the morning on waking. Mild symptoms persisted between severe attacks, and the blood chemistry remained approximately the same.

On March 12, 1930, 360 grains of lactose per day were prescribed in addition to 200 grains of calcium lactate as before. These substances were divided into three doses, given before meals. The characteristic change in the chart consists in a persistent fall of the phosphate level. The symptoms were gradually alleviated and finally disappeared on March 27.

On April 4 the blood phosphates had fallen to a level which was lower than that considered necessary. The serum calcium was not normal, and therefore the dose of lactose was reduced to 180 grains and the calcium increased to 240 grains per day. The phosphate level rose, but remained within normal limits. The serum calcium also apparently rose somewhat. The temporary rise of serum calcium on March 27 is unexplained. The average serum calcium level was not greatly affected by the addition of lactose, and except for the one high value, there was a slight lowering of this figure until the calcium intake was increased on April 4. Since March 27 the patient has been completely symptom free, with the exception of two days when she voluntarily discontinued treatment. On May 8 she stated that she was feeling better than she had for years.

CASE 2—(219959) A woman, thirty four years of age, underwent thyroidectomy for adenomata of the thyroid with tracheal compression on November 21, 1929. The basal metabolic rate before operation was minus 16 per cent. On the morning following operation she complained of tingling in the fingers. Her serum calcium on this day was 8.6 mg per 100 cc.

At first the patient received calcium gluconate one dram three times a day, and calcium lactate, 20 grains three times a day. Her symptoms demanded the administration of

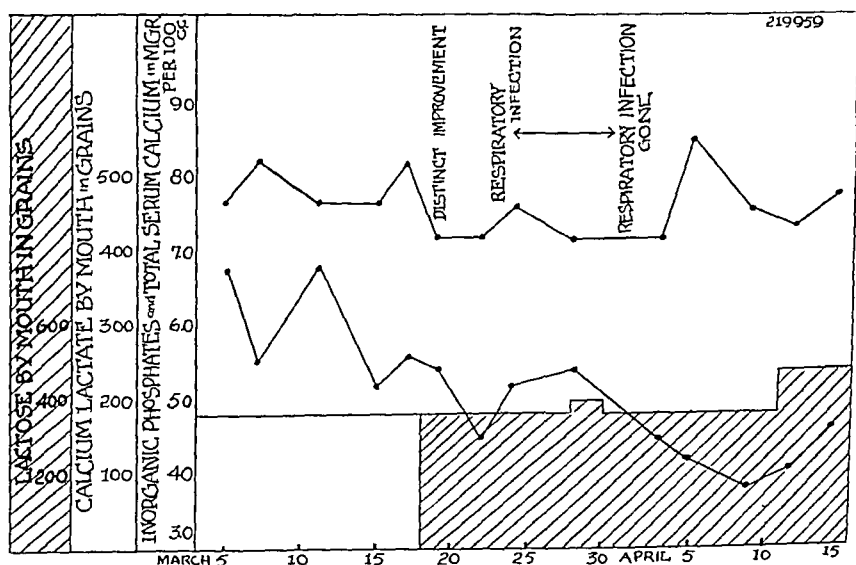


Chart 9

parathormone (parathyroid extract—Collip) on three occasions during the first thirteen days after operation, during which period the blood calcium fell to 7.7 mg. On December 4 the treatment was changed to one dram of calcium carbonate three times a day before meals. The symptoms were lessened in severity and the blood calcium rose from 7.7 mg to 8.7 mg on December 9, and to 9.2 mg on December 12.

This treatment was continued, and the patient was not again observed until she returned on March 5, complaining that she had experienced almost daily stiffness and numbness of the fingers and twitching of the facial muscles. The serum calcium was then 7.7 mg per 100 cc and blood phosphates 6.8 mg as shown at the beginning of Chart 9. The treatment was changed on this date to calcium lactate, 180 grains per day, given in three doses of one dram each before meals. The serum calcium changed very little and the blood phosphate varied between 6.8 mg and 5.2 mg per 100 cc. There were mild symptoms daily.

On March 18 the administration of lactose was started in doses of two drams three times a day before each meal. On the first day there was no improvement. On the second day the symptoms had disappeared, and on March 22 the blood phosphate was 4.5 mg and the serum calcium 7.2 mg. On March 23 she developed a mild infection of the upper respiratory tract, associated with aching pains in the back and limbs. This lasted until March 30, and was accompanied by a slight rise in the blood phosphate level, but there was no paresthesia of fingers or toes and no stiffness of the fingers. Following March 23 the blood phosphate level continued to fall, and the patient felt entirely well.

It should be noted that while the patient's serum calcium varied slightly around the level of 8 mg per 100 cc, she had moderate symptoms of tetany associated with a high blood phosphate. Later, when the average serum calcium level was lower, the symptoms were greatly improved, associated with a fall in blood phosphates. It might be mentioned again that the blood phosphate levels given were estimated while the patient was fasting. Probably they were lower during the day than those shown in the chart.

CASE 3—(223213) A woman aged thirty-two underwent a thyroidectomy for hyperthyroidism on February 20, 1930. Her basal metabolism before operation was plus 49 per cent. About thirty-six hours after the operation she complained of twitching of the facial muscles and paresthesia and stiffness of the fingers. Both Chvostek's and Trousseau's signs were present. The serum calcium on February 22 was 7.2 and on February 24 was 6.7 mg per 100 cc. The blood phosphate on this date was 6.2 mg per 100 cc.

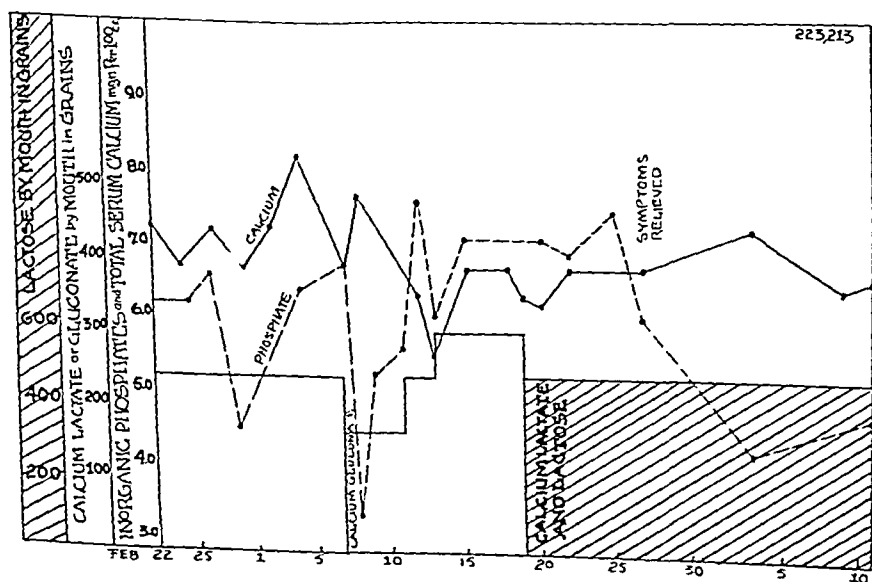


Chart 10

Treatment was begun February 22 in the form of calcium lactate, 240 grains per day, 1 dram before meals and at bedtime, as shown in Chart 10. Symptoms were present daily, and the fasting serum calcium and blood phosphate varied as indicated.

On March 7, calcium lactate was discontinued and calcium gluconate was started, 180 grains per day in doses of 1 dram before each meal. The blood phosphate content fell markedly, but soon rose again to a higher level than before, in spite of the fact that the dose of calcium gluconate was raised first to 240 grains and later to 300 grains per day. The symptoms had disappeared on March 10, but on March 11 they reappeared and were more severe than before. Symptoms were present from the latter date until March 26.

On March 19 the treatment again was changed. The same amount of calcium lactate as was given previous to March 7 was prescribed. In addition to this, lactose was given in amounts of 480 grains per day, two drams being given with one dram of calcium lactate before each meal and at bedtime. For six days no distinct benefit was noted, but on March 27, the eighth day after the administration of lactose was started, there was definite improvement in the severity of the symptoms, accompanied by a fall in blood phosphates but without any rise in serum calcium. On this treatment the blood phosphates fell to a normal level, and symptoms disappeared entirely. The fasting blood phosphates are known to have remained normal for at least one month on this treatment with the exception of two estimations done within one week following the extraction of an acutely abscessed tooth. On these occasions the phosphates were 64 and 60 mg, respectively. The patient was known to be symptom free on May 8, 1930.

It is interesting to note again that the improvement in symptoms was associated with a fall in the level of blood phosphates but not with a distinct rise in the total serum calcium.

CASE 4—(124350) A woman thirty-four years of age underwent thyroidectomy for adenoma of the thyroid in June, 1923. The second day following this operation tetany developed. She experienced tingling, numbness, and stiffness of the fingers daily, and on occasions had severe generalized convulsions which were thought to be epileptic in character, but as the convulsions have not recurred since she has had adequate treatment for tetany, it is probable that they were caused by this condition.

From June, 1923, to the present time the patient has required constant treatment. At first, when she was taking 10 grains of calcium lactate and 1/10 grain of parathyroid extract twice a day, together with a mixture containing sodium bromide and tincture of hyoscyamus, she was not relieved, and continued to have symptoms daily and generalized convulsions occasionally. In May, 1925, when the calcium lactate was increased to 20 grains three times a day, she was somewhat relieved.

In June, 1926, injections of parathyroid extract (Collip) were begun, and a dose of 1 to 2 cc was administered subcutaneously every second or third day. In addition to this, she received parathyroid extract, grains 1/5, and calcium lactate, grains 10, three times daily, together with cod liver oil. In October, 1928, the intake of calcium lactate was raised to 120 grains per day. The patient felt better than she had since before the onset of the condition, but as moderately severe symptoms frequently were present it was still necessary to give parathyroid extract (Collip) in doses of 20 to 40 units (1 to 2 cc) on alternate days.

This type of treatment was continued until January, 1930, when the dose of calcium lactate was raised to 360 grains per day. At this time the symptoms, though reduced, were persistent, and the patient began again to take about 25 cc of parathyroid extract daily. On March 11, 1930, lactose was added in amounts of 360 grains per day, with two drams each of lactose and calcium lactate before meals. The patient became symptom free, and the parathyroid extract was reduced gradually.

Many important details have been necessarily omitted, but this outline conveys some idea of the severity of the disease in this case and of the type of treatment employed. Serum calcium and blood phosphate levels are not quoted in detail, but from January, 1925, to March, 1930, the serum calcium varied from 6.2 mg to 9.7 mg per 100 cc, according to

the treatment used. The most constant figure was between 7 and 8 mg. The blood phosphates in January 1930 before beginning treatment with lactose, were 6 mg per 100 cc.

At the time indicated at the beginning of Chart 11 the patient was receiving 1 cc of parathroid extract (Collip) per day, 500 grains of calcium lactate, and 360 grains of lactose. As she had been symptom free since beginning the use of lactose, it was decided that the calcium lactate and lactose should be stopped entirely in order to see how much parathroid extract (Collip) was necessary to relieve the symptoms completely. With the patient's permission and cooperation on April 9 no calcium or lactose was taken at noon or in the evening, two drams of each being taken before breakfast as usual.

Early in the morning of April 10 symptoms appeared and therefore 2 cc of parathormone (parathroid extract Collip) were given subcutaneously at 8 A.M. and 4 cc at

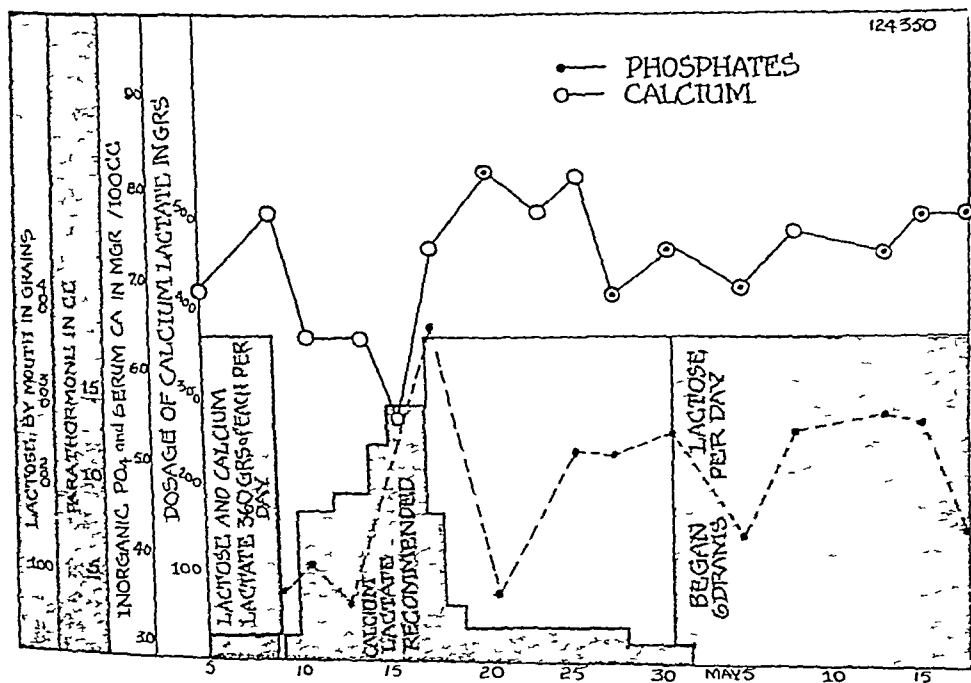


Chart 11

5 P.M. On April 11 the severity of the symptoms increased and 8 cc of parathormone were used, 4 cc at 8 A.M. and 4 cc at 5 P.M. The same dose was given on the following day. The blood calcium apparently was falling but the phosphates were being held well in check. On April 13, despite the administration of 4 cc of parathormone at 8 A.M. and 5 cc at 5 P.M. the symptoms were becoming more severe. The fingers were stiff, the patient was nauseated all day and took only small amounts of food with difficulty and she staggered markedly when she walked. On April 14 6 cc of parathormone were given subcutaneously at 9 A.M. There was slight relief in half an hour but at 4:30 P.M. the symptoms again became more severe. On April 15, 6 cc of parathormone were injected at 8 A.M. and 7 cc at 5 P.M. The patient was slightly better on this day, but still had some carpal spasm and nausea. On April 16 the symptoms were severe. The muscles of the arms and legs were becoming sore. Seven cc of parathormone were given at 8 A.M. and 6 cc at 5 P.M. The report on the blood chemistry showed that the serum calcium had fallen to 5.2 mg and the phosphate had risen to 4.5 mg per 100 cc. Because of this and the severity of the symptoms calcium lactate was given 2 drams before dinner and 2 drams at bedtime.

The following day she again began to take calcium lactate, 360 grains per day. The serum calcium rose promptly and the symptoms were completely relieved. The parathormone was reduced to 2 c.c. per day. Since slight symptoms were present, the dosage of parathormone was not decreased until April 28. The symptoms from April 17 to May 1 were very slight, but not mild enough for the parathormone to be discontinued. On May 2, 360 grams of lactose were given, divided into three doses, before meals, in addition to the same dosage of calcium lactate as before. The patient was instructed to use parathormone as before, when required for the relief of symptoms. Up to May 8, she had not found it necessary to use any injections, as there had been no paresthesia or stiffness of the fingers since administration of lactose was started.

It was expected that the phosphate level would rise somewhat after the discontinuance of the parathormone, and this has occurred. The patient has noticed very mild symptoms on two days, but they have been so slight that up to the present time she has preferred not to use parathormone.

The patient was last seen on May 20, when she was practically symptom free.

DISCUSSION

In chronic tetany, definite improvement in symptoms apparently results from the feeding of lactose. This is associated with a fall in the level of inorganic phosphates in the blood. During glucose assimilation after the ingestion of glucose or other carbohydrates, there is always temporary improvement in symptoms, associated with a fall in the phosphate level. Nevertheless, even frequent carbohydrate feedings do not result in the permanent benefit which is observed after the administration of lactose. After single doses of glucose or lactose the blood phosphate returns to the previous level within four hours. It seems remarkable that when lactose is used therapeutically, low levels of inorganic phosphate can be demonstrated in the blood twelve to fourteen hours after the last dose of lactose is taken.

The complete mechanism of the action of lactose on blood phosphates is obscure. At first it was thought that the slow digestion of lactose, and the resulting slow absorption of glucose and galactose, might account for the prolonged depression in the phosphate level in the blood. After examination of the blood following the oral administration of one dose of lactose this seems unlikely. The blood sugar level rises sharply and falls again within four hours, indicating rapid absorption and assimilation. The inorganic phosphates also return to a normal level within this period. The feces have not been examined quantitatively, but it has been shown that the phosphate depression is not the result of increased urinary excretion. On the contrary, there is a retention of urinary phosphates after the administration of single doses of lactose.

The possibility that galactose might have a specific effect was considered. It is known that glucose can be formed in the body from galactose which has been absorbed from the intestine. If the glucose, so formed, is assimilated in the same manner as ingested glucose, it was thought that an extended period of carbohydrate assimilation might result. This in turn would cause a prolonged depression of the phosphate level which would simulate the long, low phosphate curve noted after the administration of glucose to diabetics, when assimilation of the glucose is delayed.

At present, the data concerning this point are insufficient to warrant definite conclusions. Our results, however, and those of Barrenscheen²⁵ fail to demon-

strate any connection between the metabolism of galactose and that of inorganic phosphate in the blood. The use of galactose over long periods may cause us to draw different conclusions. As yet, there is no definite evidence that the efficacy of lactose is due to changes in intermediary metabolic processes. It may be that there is a slowing of the absorption or an acceleration of the excretion of the phosphates.

Neither Dragstedt¹ nor his recent supporter, Hutton,² have associated the effect of lactose with mineral metabolism. Those who believed that parathyroid tetany was caused by changes in calcium metabolism were of the opinion that lactose produced an increased absorption of calcium by the production of acidity in the gut.²⁶ So far as we are aware, no one has demonstrated an increased blood calcium after lactose administration. Since calcium phosphate is very insoluble, it is possible that an increased absorption of calcium might result in a lowering of the phosphate level due to a deposition of calcium phosphate in the tissues. Hence, increased calcium absorption might not be apparent on examination of the blood.

SUMMARY AND CONCLUSIONS

1 The symptoms of chronic parathyroid tetany may be lessened in severity or completely controlled by lowering of the amount of inorganic phosphates in the blood without raising the total calcium content of the blood serum.

2 Glucose temporarily lowers the amount of inorganic phosphates in the blood of normal subjects and in individuals with chronic parathyroid tetany.

3 Lactose in single doses has the same effect as glucose.

4 Galactose in single doses has no effect on the inorganic phosphate content of the blood.

5 After ingestion of glucose or of lactose there is a decrease in the amount of phosphate excreted in the urine. This does not occur after the administration of galactose.

6 Three cases of chronic parathyroid tetany are reported in which symptoms were present in spite of the oral administration of large doses of calcium lactate. The addition of lactose resulted in complete relief, associated with a lowering of the phosphate content of the blood.

7 One case of chronic parathyroid tetany is reported in which large doses of calcium lactate alone failed to give complete relief and very large doses of parathyroid extract (Collip) alone failed to give relief. The symptoms could be controlled by the oral administration of large doses of calcium lactate, together with the subcutaneous injection of parathyroid extract. Calcium lactate in large doses in combination with lactose gave relief without the addition of parathyroid extract.

8 The mechanism of the action of carbohydrates in lowering the amount of phosphates in the blood is discussed.

We are greatly indebted to Miss Louise Van Alstine for her technical assistance throughout the work.

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A COMPARISON OF THE VASOCONSTRICTIVE ACTION OF ADRENALIN AND EPHEDRINE ADDED TO THE LOCAL ANESTHETIC SOLUTION*

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IN THE introduction of adrenalin, local anesthesia was given a drug the local application of which causes a contraction of terminal blood vessels, rendering the tissues bloodless and causing an increase of the local anesthetic action and diminishing the general toxic action. It is, thus, of importance because of its ischemia producing properties. Adrenalin is not an anesthetic agent itself but local action of other drugs is made more intense and lasting when combined with it. The extent of the anemia of tissues and anesthesia are independent of one another. The first depends upon the adrenalin content, while the latter depends upon the quantity of local anesthetic drug in the solution. Diluted solutions of anesthetic substances can thus be made to produce a more intense effect of longer duration by the addition of adrenalin. The limits of usefulness of local anesthesia have thus been materially increased, the results are more certain, the technic in many instances has been simplified, and danger from certain operations has been markedly reduced.

The dose of adrenalin should be carefully considered, as large doses are very dangerous. The greater the concentration the greater the toxic action. The chief toxic symptoms are palpitation of the heart, depression, and often a feeling of dread or alarm, difficult respiration, a sense of constriction across the chest and a fullness and throbbing in the head. These symptoms, accompanied by rapid rise in blood pressure, usually pass off in a few minutes, when the drug should be more cautiously used. Operators often blame the local anesthetic for such symptoms occurring in the course of an operation, when the adrenalin is the disturbing agent.

In the strengths ordinarily employed, its use is not followed by any after reaction. There is no hyperemia, but the tissues gradually resume their normal vascularity, and there seems to be no retarding or injurious action upon the healing of wounds. When used in fairly large doses, it seems that there is often more after pain in the wound than would have been the case under ordinary conditions. If an excessive dose is used, there often follows a primary anemia which is marked and prolonged, a vasomotor paralysis, during which time the vessels remain dilated with open mouths, and secondary hemorrhages may occur.

Following the use of excessive doses the resulting anemia may be so profound and prolonged as to lead to death of a tissue with sloughing. The author has found that for ordinary surgical work the addition of ten drops to 100 c.c. of anesthetic solution usually suffices. The dose should vary according to the age and condition of the patient. Childhood, old age, arteriosclerotics,

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and those suffering from lesions of the vascular system, high blood pressure, Grave's disease, diabetes, and others, are more susceptible to its influence. The dose in these cases should be lessened accordingly, and often should not be used at all.

For many years adrenalin has been the sole sympathomimetic (i. e., producing effects similar to the result of excitation of sympathetic innervations) drug in clinical use. Recently, its sole competitor, ephedrine, has been introduced. The rise of ephedrine from obscurity to its present state of wide spread popularity involves several features of unusual interest. In a short space of time it has found clinical favor in the treatment of bronchial asthma, hay fever, bronchitis, Adams-Stokes syndrome, combatting the fall in blood pressure in spinal anesthesia, shrinking the congested nasal mucous membrane, and in dilating the pupil for ophthalmic examinations.

Ephedrine produces vasoconstriction as determined by perfusion experiments on the frog's leg, the ear of a rabbit, and the intestine, spleen, and kidney of the dog. This action, like that of adrenalin, is essentially peripheral, and not dependent upon stimulation of the vasomotor center or other parts of the central nervous system. Both experimental and clinical data show, however, that as a vasoconstrictor, it is much less powerful and uniform in its effects than adrenalin.

More recently various combinations of adrenalin and ephedrine have found favor in clinical use. Adrenalin applied to mucous membranes has a prompt and powerful vasoconstricting action upon the capillaries reducing congestion and turgescence of the tissues. This action, however, is of relatively short duration. Ephedrine is added to supplement the action of adrenalin, prolonging the astringent effect materially. Hypodermically administered, the advantage is that the action is more lasting than that of adrenalin alone. Schaumaun has found that very small amounts of adrenalin augment the constrictor action of ephedrine in experimental work.

No adequate information seems available on the comparative vasoconstrictive action of adrenalin and ephedrine when added to the local anesthetic solution. This has constituted one of the most important uses of adrenalin and thus far ephedrine has not entered this field of usefulness. Since the systemic action of ephedrine is less intense, it occurred to the author that it might be a more suitable local anesthetic adjuvant in those cases of thyrotoxicosis, hypertension, and cardiopathies, in which adrenalin is poorly tolerated.

The fact that the action of ephedrine is more prolonged than that of adrenalin, also suggests a possible advantage in adding a combination of the two to the local anesthetic solution for a more prolonged local anesthesia. This would thus be similar to the employment of the combination of adrenalin and ephedrine in rhinology, and in hypodermic use. In order to solve this question the following work was carried out.

METHOD

The anesthetic power of all combinations was determined by dermal wheals on the human skin. This is an attractive method because it parallels clinical usage. It involves direct action on the terminal nerve filaments and sensory

end organs of the skin. Anesthesia is but very little dependent upon pressure within the layers of skin, because control wheals of physiologic salt solution do not produce anesthesia. Anesthesia therefore results from a direct chemical action upon the nerve endings which may be prolonged by a vasoconstrictive drug by retarding absorption.

TECHNIC

The anterior surfaces of the thighs were closely shaved. Dermal wheals were then raised with a special local anesthesia syringe and finest hypodermic needle. The needle was thrust beneath the skin surface with bevel downward. At the moment the needle point entered the epidermis injection began, which was always endermic and not subcutaneous.

The area of wheals was estimated as the size of a dime and required 0.8 to 1 c.c. of solution each. It is important that all wheals be as nearly the same size as possible and contain the same amount of solution, all of which has been injected intracutaneously. Adequate controls were employed so that no disturbance of sensation except that of complete abolishment of sensation was interpreted as anesthesia.

All wheals were made upon the writer by himself. The skin of the thighs is of such thickness that accurate wheals may be raised painlessly when the substance is anesthetic. The sensitiveness of the skin and the rapidity of absorption vary in different areas of the body. These features also vary in different individuals, depending upon familial traits, exposure, vocation, etc. By employing the same skin area in the same individual, these factors remain constant. The duration of anesthesia in the same cutaneous area may also be shortened by previous brisk massage, heating or muscular exercise because of the improved circulation and consequently more rapid absorption. In these tests the subject remained seated and sources of external heat were avoided. Wheals were marked with a circle of mercurochrome as soon as raised, so that the center of the endermic infiltration was easily identified for testing after the wheal had disappeared. Tests for sensation were made by scratching the area with a wooden applicator or with a needle, as is done in vaccination.

The anesthetic drugs employed were novocaine (Metz) and neothessin (Lilly). Adrenalin chloride solution 1:1000 with chlorotone added as a preservative, as furnished to the trade by Parke Davis & Company, was used. A supply of ephedrine hydrochloride was furnished by the Research Laboratories of Eli Lilly & Company especially for these tests. In addition, the 3 per cent ephedrine solution with 0.5 chlorotone as a preservative as furnished to the trade by Abbot was available. Chlorotone itself possesses local anesthetic properties in 0.5 per cent strength, but not in the high dilution of 10 drops of the stock ephedrine-chlorotone solution to 100 c.c. of salt solution.

Table I, first line, shows the anesthetic power of novocaine alone both by duration of anesthesia in minutes and minimal anesthetic concentration. All figures represent the average of many trials. The second line shows the prolongation of anesthesia by the addition of 10 drops of 1:1000 adrenalin solution to 100 c.c. of anesthetic solution. In all these tests the duration was more than twice that of the anesthetic solution alone.

TABLE I
ADRENALIN AND EPHEDRINE

Per cent Strength	DURATION OF ANESTHESIA IN MINUTES						
	1/2	1/4	1/8	1/16	1/32	1/64	1/128
Novocaine alone	23	16	10	6	?	0	0
Novocaine and adrenalin	64	49	30	10	3 ?	0	0
Novocaine and ephedrine	25	21	16	12	4 ?	0	0
Novocaine and adrenalin plus ephedrine	65	52	35	12	3 ?	0	0

Ten drops of 3 per cent ephedrine solution were next added, results of which are shown in the third line. A comparison of these values with those of novocaine alone shows but a slight increase, although the concentration is 30 times that of adrenalin in line two.

The last line shows values for the combination of adrenalin and ephedrine added to the anesthetic solution. It will be observed by comparison with the second line that there is no advantage by the addition of ephedrine in thirty times the amount of adrenalin.

The same series of tests were employed with neothessin (Lilly) as the local anesthetic agent, results of which are expressed in Table II. An inspection of

TABLE II
ADRENALIN AND EPHEDRINE

Per cent Strength	DURATION OF ANESTHESIA IN MINUTES						
	1/2	1/4	1/8	1/16	1/32	1/64	1/128
Neothessin alone	27	24	18	15	9	6	?
Neothessin and adrenalin	63	60	52	30	22	15	6 ?
Neothessin and ephedrine	35	23	20	15	10	7	?
Neothessin and adrenalin plus ephedrine	66	55	41	28	25	12	?

these data corroborates in all respects the facts brought out in Table I. There is no prolongation of local anesthetic action by the addition of ephedrine in thirty times the concentration of adrenalin commonly employed. Neither is there any advantage in the combination of adrenalin and ephedrine as an adjuvant to the local anesthetic solution.

COMMENT

While it is to be admitted that ephedrine has a slight vasoconstrictor action, it is very weak indeed and only serves to emphasize the fact that adrenalin is one of the most powerful drugs known. The results of experimental investigators differ widely. Thus Amatsu and Kubota observed constriction of frogs' legs upon perfusion with a 1 to 10,000 solution of ephedrine hydrochloride. The vessels of the ear of the rabbit were constricted by solutions ranging from 1 to

2000 to 1 in 20,000 Loo and Read working with toads found a 1 in 20,000 solution effective Other workers while agreeing that ephedrine is a vasoconstrictor found it a much less powerful one than these results would indicate Thus Kreitmar found constriction of frogs' vessels with a 1 in 10 dilution of ephedrine, but none with a 1 in 100 solution Gradinesco found only slight constriction with a 1 in 100 solution These differences are confusing and seem entirely too great to fall within the realm of experimental error It was, therefore decided to titrate the vasoconstrictive power of ephedrine against that of adrenalin, by comparing dilutions of each required to prolong similar strength local anesthetic solutions an equal interval of time The amount of ephedrine added was thus doubled until equal amounts of 3 per cent strength ephedrine and 1 per cent novocaine were used In no instance was there prolongation of anesthesia In fact the higher amounts diminished the duration, indicating an incompatibility when the amount of ephedrine added was too great

CONCLUSIONS

1 The peripheral vasoconstrictive power of adrenalin is strikingly demonstrated by the prolongation of anesthesia when very small amounts are added to the local anesthetic solution This action has earned for it the name of "chemical tourniquet"

2 Ephedrine, its only competitor in general clinical use cannot be shown to possess any vasoconstrictive power at all by the dermal wheal method on man

3 No value can thus be demonstrated by its combination with adrenalin as an adjuvant to the local anesthetic solution

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STUDIES IN THE SEROLOGY OF SYPHILIS*

VII ON THE SUPPOSED ARTIFICIAL INDUCTION OF A POSITIVE WASSERMANN REACTION IN ORIGINALLY NEGATIVE HUMAN SERA

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IT IS commonly believed that the positive Wassermann test observed in syphilis reflects an obscure physicochemical change in the serum colloids, rather than the appearance of a new specific substance. This theory is apparently borne out by the reputed ease of inducing positive Wassermann reactions in originally negative serum by such simple procedures as shaking, acidification, dilution, etc.

As will be shown in the present paper, however, most of the observations which seem to confirm this concept of the Wassermann as a labile and altogether anomalous reaction, are based upon errors of technic and interpretation. Far from being readily induced in normal sera by the most diverse nonspecific processes, it is difficult if not impossible to produce a positive Wassermann in originally negative human serum by such simple physical or chemical treatment.

It will be further shown that the majority of the procedures reputed to induce a positive Wassermann reaction make the serum somewhat anticomplementary. The supposed positive reaction is in reality a summation of two anticomplementary effects, that of the antigen and that induced in the serum by the preliminary treatment. An example will make this clear. The Wassermann reaction actually measures the amount of complement which remains free after the interaction of complement, antigen and serum. If this residual quantity is sufficient to cause hemolysis, the reaction is negative, but if so much has been destroyed that the remaining quantity does not suffice to hemolyze sensitized cells, the reaction is adjudged positive. The exact proportion of complement which must be destroyed in order to obtain a positive reaction varies with the technic used. Let us assume it to be 60 per cent, as it is in a technic using 2 to $2\frac{1}{2}$ units of complement. At the time when most of these artificial positives were reported, the amount of antigen used in the test was so close to the completely anticomplementary quantity that it must have destroyed considerable complement, even though not enough to prevent hemolysis entirely. Thus, Hirschfeld and Klingner (1914) used antigen in one-half of its completely anticomplementary quantity. As is illustrated in Protocol 1 and Table I, the antigen in such a set-up may destroy fully half the amount of complement which must disappear in order to give a positive Wassermann reaction, i. e., 25 to 40 per cent in the hypothetical case cited.

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PROTOCOL 1

Dried powdered beef heart (Difco) was extracted for three days with 95 per cent alcohol (5 cc per gram). The filtered extract was diluted with 4 volumes of NaCl N/7 (0.85 per cent), and serially decreasing quantities of the dilution incubated with complement, as indicated in the following set up

Antigen 1.5, cc	0.2	0.1	0.05	0.025	0.0125
NaCl N/7, cc	0.2	0.3	0.35	0.375	0.3875
Guinea pig complement, 1:10, cc	0.2	0.2	0.2	0.2	0.2

After four hours' incubation at 0° to 6° C, and one half hour at 37° C, 0.4 cc of a sheep cell suspension sensitized with 4 units of rabbit antioceptor were added to each tube and the time necessary for complete hemolysis was noted. As has been shown elsewhere (Eagle, 1929), the hemolysis time is an accurate measure of the amount of complement destroyed. The results of many experiments, illustrated in Table I, show conclusively that half, and even one fourth, of the completely anticomplementary quantity of antigen destroys a significant quantity of complement, even though sufficient remains free to allow for hemolysis in half an hour.

TABLE I

SHOWING THAT FRACTIONS OF THE "ANTICOMPLEMENTARY" QUANTITY OF ANTIGEN DESTROY SIGNIFICANT AMOUNTS OF COMPLEMENT

Antigen 1.5 cc	0.2	0.15	0.1	0.075	0.05	0.375	0.025	0.0187	0.0125
Hemolysis	0	0	±	±	±	±	-	±	±
% complement destroyed	90	90	70	60	50	35	30	-	-

If now, by shaking, the serum is also made somewhat anticomplementary (e.g. 30 to 40 per cent) the total complement destroyed is sufficient to prevent hemolysis and one has a pseudopositive result. This is not a fixation by a lipid-reagin compound, as is the true positive Wassermann reaction, but a destruction of complement by an anticomplementary serum plus an anticomplementary antigen. This summation of anticomplementary effects is similar to the irreversible destruction caused by immersing the tube in a water-bath at a temperature of 56° C and quite unlike complement fixation as given by syphilitic serum. The evidence for this contention is given in the following sections.

I

The experimental procedures here listed have been supposed by various investigators to produce a positive Wassermann reaction *in vitro* in originally negative serum. *Shaking* (Hirschfeld and Klinger 1914), *acidification* (Nathan 1920, McMeans 1923), *dilution* (Nathan, 1918; Forssmann 1921), *adsorption* with a particulate suspension (Hirschfeld and Klinger, 1914), chemical treatment, as by *amino acids* (Bachmann 1922), *ether* (Forssmann, 1921), and *agar* (Hirschfeld and Klinger 1914), *aging* (Rabinowitsch 1914) etc.

Serum from nonsyphilitic human subjects was treated by these methods (Protocol 2) and subsequently tested for its anticomplementary and Wassermann titers (Protocol 3). The results obtained with a single negative serum are summarized in Table II. Qualitatively similar results were obtained with five lots of pooled serum from nonsyphilitic human beings.

PROTOCOL 2

Methods of treating fresh normal human serum preparatory to studying its anticomplementary and complement fixing properties

1 *Shaking*—Serum was diluted 1:10 in (a) water and (b) NaCl N/7 (0.85 per cent) and shaken in a shaking machine at 240 "to and fro" movements per minute for five hours. The solution gradually becomes cloudy due to the denaturation of a portion of the serum globulin, particularly in the aqueous dilution.

2 *Acidification*—HCl N/5 was added to serum until the point of normal precipitation was reached (P_H 5.055). After five hours at room temperature, part of the serum was exactly neutralized by the addition of NaOH N/5.

3 *Dilution*—Serum was diluted 1:10 with H₂O and allowed to stand for five hours at room temperature.

4 *Adsorption with a particulate suspension*—(a) Kriohn was added to normal serum, the mixture shaken for one minute, and after standing for one hour at room temperature, centrifuged. Serum was similarly treated with normal and specifically agglutinated suspensions of *B. typhosus*, *B. coli*, *Pneumococcus I*, and sheep red cells, and with the precipitate obtained by adding sheep plasma to a rabbit antiserum.

5 *Glycocoll, ether and agar*—Serially increasing quantities of these substances were added to normal serum, and the mixtures allowed to stand for four hours at room temperature. The ether serum mixtures were tested both by layering the ether onto the serum and allowing it to evaporate, as well as by thorough emulsification of the mixture.

6 *Ageing*—Sterile negative serum was kept at icebox temperature for one, two and four months.

PROTOCOL 3

Method used to test the anticomplementary action of the treated sera and their behaviour in the Wassermann reaction.

Anticomplementary Titer—The sera treated as described in Protocol 2 were inactivated at 56° C for twenty minutes. To varying quantities of the treated sera in a total volume of 0.8 cc were added 0.4 cc of 1:10 guinea pig complement, and the mixture placed at 4° to

TABLE II

TREATMENT OF SERUM	CC SERUM USED	PER CENT COMPLEMENT DESTROYED BY		
		SERUM ALONE (ANTICOMPLE- MENTARY)	SERUM + ANTIGEN (ANTICOMPLE- MENTARY + FIXATION)	SPECIFIC INTERACTION OF SERUM WITH ANTI- GEN (WASSERMANN)
Original serum	0.4	25	25	0
	0.2	<10	<10	0
	0.1	<10	<10	0
	0.05	<10	<10	0
	0.025	<10	<10	0
Shaken with NaCl N/7	0.4	65	60	0
	0.2	50	50	0
	0.1	30	35	0
	0.05	15	15	0
	0.025	<10	<10	0
Shaken with H ₂ O	0.4	>90	>90	—
	0.2	>90	>90	—
	0.1	90	>90	—
	0.05	75	80	0
	0.025	45	30	0
Acidified	0.4	>90	>90	—
	0.2	>90	>90	—
	0.1	70	70	0
	0.05	40	40	0
	0.025	20	20	0

TABLE II (Continued)

TREATMENT OF SERUM	C C SERUM USED	PER CENT COMPLEMENT DESTROYED BY		
		SERUM ALONE (ANTICOMPLEMENTARY)	SERUM + ANTIGEN (ANTICOMPLEMENTARY + FIXATION)	SPECIFIC INTERACTION OF SERUM WITH ANTIGEN (WASSERMANN)
Acidified and Neutralized	0.4	55	60	0
	0.2	35	40	0
	0.1	20	20	0
	0.05	<10	<10	0
	0.025	<10	<10	0
Dilution	0.4	85	80	0
	0.2	55	50	0
	0.1	30	30	0
	0.05	<10	<10	0
	0.025	<10	<10	0
Shaken with Kaolin	0.4	30	30	0
	0.2	15	15	0
	0.1	<10	<10	0
	0.05	<10	<10	0
	0.025	<10	<10	0
Shaken with killed B Typhosus	0.4	20	20	0
	0.2	<10	<10	0
	0.1	<10	<10	0
	0.05	<10	<10	0
	0.025	<10	<10	0
Shaken with ag glutinated B Coh	0.4	90	90	0
	0.2	75	80	0
	0.1	60	60	0
	0.05	55	50	0
	0.025	30	30	0
Shaken with sheep anti sheep serum ppt	0.4	>90	>90	-
	0.2	>90	>90	-
	0.1	70	75	0
	0.05	60	60	0
	0.025	40	40	0
Shaken with thick sheep cell suspen sion*	0.4	20	20	0
	0.2	<10	<10	0
	0.1	<10	<10	0
	0.05	<10	<10	0
	0.025	<10	<10	0
Shaken with ag glutinated* sheep cells	0.4	>90	>90	-
	0.2	>90	>90	-
	0.1	85	80	0
	0.05	60	50	0
	0.025	40	30	0
In a similar manner, aged serum, and serum treated with glycozell, ether, or agar gives exactly the same amount of complement fixation without antigen as it gives with antigen, the serum is Wassermann negative				
A syphilitic serum	0.4	25	>90	>90
	0.2	<10	>90	>90
	0.1	0	>90	>90
	0.05	0	>90	>90
	0.025	0	>90	>90
	0.0125	0	>90	>90
	0.006	0	>90	>90
	0.003	0	70	70

*Heated serum was shaken with the sheep cell suspensions in order to prevent any hemolysis due to native amboceptor and native complement

8° C for four hours, followed by one half hour at 37° C. Eight tenths c.c. of sheep cells sensitized with four units of amboceptor were then added, and the time required for complete hemolysis was noted. As has been shown elsewhere (Eagle, 1929), the amount of complement remaining free, and therefore the amount destroyed, can be accurately calculated by measuring this hemolysis time.

Wassermann Titer—Simultaneously, a quantitative Wassermann reaction, i.e., the determination of the amount of complement fixed and destroyed by the serum in the presence of beef heart lipid (antigen) was carried out, using the same amounts of complement and the same amount of sensitized sheep cells, in the same total volume under the same conditions. Care was taken to use an antigen which is not in the slightest anticomplementary in the quantity used (0.4 c.c. of a 1:60 dilution in NaCl N/7 of a beef heart antigen containing 0.6 per cent cholesterol, anticomplementary unit 1:5 dilution). The results are summarized in Table II. At the bottom of the table the results obtained with a known syphilitic serum are given for contrast.

Comparing the results obtained by these two series of tests it is found that the amount of complement destroyed by all the treated sera in the presence of beef heart lipid is, within the limit of experimental error, the same as is destroyed by the treated sera alone, they are completely Wassermann negative. The various manipulations have succeeded only in making the serum somewhat anticomplementary.

II PSEUDOPPOSITIVE WASSERMANN REACTIONS WITH NONSPECIFIC ANTIGENS

If now, using these more or less anticomplementary sera, one tries complement fixation with a somewhat anticomplementary antigen, the summation of complement destruction by serum and by antigen may, and sometimes does, simulate the phenomenon of complement fixation as given by syphilitic serum. Actually, however, there has been no complement fixation by a reagent antigen compound, such as determines the positive Wassermann reaction. Substances other than a Wassermann antigen will produce the same pseudoreaction, e.g., weakly anticomplementary suspensions of lecithin, of milk lipid, of sheep cell lipid, of cholesterol, or a weak solution of HCl none of which has the slightest specific reactivity with human syphilitic serum, serve equally well to give this pseudo fixation of complement (Protocol 4 and Table III).

PROTOCOL 4

The preparation of lipid "antigens" possessing no specific reactivity with syphilitic serum

1 Lecithin (Merck) was dissolved in 95 per cent alcohol to form a 2 per cent solution this was fortified with 0.6 per cent cholesterol

2 Skimmed milk powder was extracted twice with ether (4 c.c. per gram) the dry residue was extracted with 95 per cent alcohol for three days. The alcoholic extract was then fortified with 0.6 per cent cholesterol

3 Citrated sheep's blood was washed in 10 volumes of NaCl N/7. The sedimented cells were dried at 60° to 90° C in a current of dry air not quite to dryness, and the semisolid mass was extracted with alcohol (5 c.c. per gram). The yellow extract was fortified with 0.6 per cent cholesterol

4 Human blood clots were similarly treated

5 Cholesterol was dissolved in alcohol as a 0.6 per cent solution. None of these 5 antigens have the slightest selective reactivity with syphilitic serum

The preparation of a highly anticomplementary Wassermann antigen

1 Beef heart powder (Difco) was extracted with 95 per cent alcohol (5 c.c. per gram)

for four days. The extract was fortified with 0.6 per cent cholesterol and 0.4 per cent sitosterol. Such an extract, although very sensitive, is highly anticomplementary.

The preparation of a sensitive Wassermann antigen with practically no anticomplementary properties.

Beef heart powder (Difco) was extracted from four times with ether (4 c.c. per gram) at 37° C, each extraction lasting fifteen minutes. The ether extracts, containing >95 per cent of the anticomplementary factors, were discarded, and the dried residue extracted with 95 per cent alcohol. The alcoholic extract was concentrated to one half its original volume, and sensitized as described in the preceding paragraph. Such an antigen is only slightly more anticomplementary than pure alcohol.

Complement fixation by these "antigens" and chemically or physically altered negative serum.

To normal human serum treated as described in Protocol 2 were added complement and various quantities of the antigens described in the preceding section. The amount of complement destroyed by (1) the serum alone, (2) the antigen alone, and (3) by a mixture of the serum and the antigen was then determined by the method described in Protocol 1 after four

TABLE III

PSEUDOPPOSITIVE COMPLEMENT FIXATION BETWEEN THE PARTIALLY ANTICOMPLEMENTARY TREATED SEPA AND PARTIALLY ANTICOMPLEMENTARY NONSPECIFIC ANTIGENS

		ANTICOMPLEMENTARY TITRE (NO ANTIGEN)		PSEUDOCOMPLEMENT FIXATION WITH LECITHIN AS ANTIGEN					
		% COMP DESTROYED BY SERUM ALONE	READING OF FIXATION	1 8000		1 4000		1 2000	
				% COMP DESTROYED	READING OF FIXATION	% COMP DESTROYED	READING OF FIXATION	% COMP DESTROYED	READING OF FIXATION
Serum diluted and shaken with H ₂ O c.c.	0.4	>90	-	>90	-	>90	+	>90	-
	0.2	90	-	>90	+	>90	+	>90	+
	0.1	70	±	75	±	>90	-	>90	-
	0.05	30	0	30	0	80	-	>90	-
	0.025	15	0	20	0	60	0	90	-
	0.0125	-	0	-	0	55	0	80	-
	0.0062	-	0	-	0	40	0	70	±
	0.0031	-	0	-	0	45	0	70	±
	0 (antigen alone)	-	0	-	0	40	0	70	±
Normal serum shaken with agglutinated staphylococci	0.4	>90	-	85	-	>90	-	>90	-
	0.2	75	±	75	±	>90	+	>90	-
	0.1	70	±	70	±	90	±	>90	-
	0.05	60	0	60	0	75	±	>90	-
	0.025	30	0	50	0	60	0	90	-
	0.0125	15	0	25	0	60	0	75	±
	0.0062	-	0	-	0	50	0	65	±
	0.0031	-	0	-	0	45	0	65	±
	0 (antigen alone)	-	0	-	0	50	0	65	±
Syphilitic serum	0.4	40	0	40	0	70	0	80	-
	0.1	-	0	-	0	40	0	70	±
	0.01	-	0	-	0	40	0	70	±
	0.005	-	0	-	0	40	0	70	±
	0.001	-	0	-	0	40	0	70	±
	0.0001	-	0	-	0	40	0	70	±
	0 (antigen alone)	-	0	-	0	40	0	70	±

- Indicates complete fixation, no hemolysis

± Indicates incomplete fixation, partial hemolysis

- Indicates complete hemolysis

hours at 4° to 8° C and one half hour at 37° C. The results obtained with two types of treated serum, with lecithin as antigen are given in Table III. Qualitatively similar results were obtained with each of the others.

As is illustrated in Table III, the amount of complement destroyed by mixtures of serum and any of these "antigens" is simply the sum of the amount destroyed by each alone. There is in no case true complement fixation such as is given by a mixture of syphilitic serum and beef heart lipoid. One of these "antigens" may be partially anticomplementary in the quantity used and yet allow hemolysis to take place in half an hour (a "negative" antigen control). Similarly, a physically or chemically altered serum may be partially anticomplementary and nevertheless allow hemolysis in the quantity used. Yet the same quantities of serum and of "antigen" when combined may destroy enough complement to prevent hemolysis in half an hour and give a reaction simulating the specific positive Wassermann. It is interesting to note that the anticomplementary extract of beef heart gave this type of false positive reaction with the sera altered by the above mentioned treatment, while an antigen prepared from the same lot of beef heart and possessing just as great reactivity with syphilitic serum, but without the anticomplementary properties of the other antigen, gave completely negative results.

One can readily understand why these artificially "positive" sera fail to give the precipitation reactions for syphilis (Nathan 1920), for these tests predicate an actual combination between antigen lipoids and syphilitic antibody, and merely making a serum anticomplementary would not affect a reaction in which no complement is used.

III THE COMPLEMENT FIXING PROPERTIES OF GLOBULIN DERIVED FROM FRESH SERUM

We have found only one procedure which induces a true positive Wassermann reaction in originally negative human serum. This is the precipitation of globulin from fresh serum as reported by Foissman (1921). Redissolved in NaCl N/7, this precipitate gives weak but definitely positive complement fixation with beef heart lipoids. This observation, however, does not prove the anomalous nature of the Wassermann substance (reagin), because unlike syphilitic serum, the globulin solution gives complement fixation with almost any finely dispersed lipoidal suspension. As is shown in Table IV, such a globulin solution reacts strongly with an alcoholic extract of sheep or human red cells, of milk, and with an alcoholic solution of lecithin and cholesterol, none of which reacts specifically with syphilitic serum.

PROTOCOL 5

The reactivity of globulin derived from normal fresh human serum with diverse lipoidal suspensions

To 5 c.c. of fresh Wassermann negative human serum were added 45 c.c. of cold H₂O, CO₂ gas was bubbled through the dilution for five minutes. The turbid suspension was then centrifuged, the supernatant fluid discarded, and the globulin precipitate redissolved in 5 c.c. of NaCl N/7. The solution was then tested for (1) anticomplementary titer, and (2) complement fixing reactivity by the technique described in Protocol 3, using the antigens described in Protocol 4. Care was taken to use a dilution of the antigen which is not demonstrably anticomplementary ($\frac{1}{8}$ of the anticomplementary quantity). The results are summarized in Table IV.

TABLE IV
 REACTIVITY OF THE Fresh Normal Human Serum With Various Dilutions

DILUTION ()	ANTICOMPLEXIN TITER (NO ANTIGEN)		CHOLESTEROL ALCOHOL EXTRACT OF BILE FLUID 1:10		CHOLESTEROL 1% SOLUTION 1:100		YOLKIN 1% SOLUTION 1:9000		CHOLESTEROL MILK EXTRACT		CHOLESTEROL ALCOHOL EXTRACT 1:800		CHOLESTEROL ALCOHOL HUMAN EXTRACT 1:100	
	% COMP FIXED	READING OF FIXATION	% COMP FIXED	READING OF FIXATION	% COMP FIXED	READING OF FIXATION	% COMP FIXED	READING OF FIXATION	% COMP FIXED	READING OF FIXATION	% COMP FIXED	READING OF FIXATION	% COMP FIXED	READING OF FIXATION
0.1	>90	+	>90	+	>90	+	>90	+	>90	+	>90	+	>90	+
0.2	>90	+	>90	+	>90	+	>90	+	>90	+	>90	+	>90	+
0.1	90	+	>90	+	>90	+	>90	+	>90	+	>90	+	>90	+
0.05	50	0	>90	+	>90	+	>90	+	>90	+	>90	+	>90	+
0.025	25	0	>90	+	90	+	>90	+	>90	+	>90	+	>90	+
0.0125			>90	+	10	0	90	+	90	+	>90	+	>75	+
0.0062			90	+	20	0	70	±	50	0	>90	+	10	0
0.0031			60	±	-	0	15	0	0	0	90	+	0	0
0.0016			30	0	-	0	-	0	0	0	65	±	0	0
Approximate Titer in Complete Fixing Units per cc		200	30	160	120	180	80							
Titer of a strongly positive syphilitic serum with these antigens		500	0	0	0	0	0							

+ indicates complete fixation no hemolysis
 ± indicates incomplete fixation partial hemolysis
 - indicates complete hemolysis

The globulin solution thus contains protein which reacts with apparently any properly dispersed lipid. It can therefore not be considered to represent artificially created Wassermann reagin, which reacts with a certain particular tissue lipid, and not with cholesterol, milk lipid, red cells lipid, lecithin, etc.

SUMMARY AND DISCUSSION

We have been unable to make an originally negative human serum Wassermann positive by physical or chemical treatment. As described in the text, most of the manipulations reputed to induce such an artificial positive reaction succeed only in making the serum anticomplementary. It is true that the globulins precipitated from fresh sera are Wassermann positive insofar as they give complement fixation with beef heart lipid, but unlike syphilitic serum, this globulin solution reacts equally well with milk lipid and egg lecithin, with cholesterol and an alcoholic extract of sheep or human red cells, and presumably, with any colloidal lipid suspension. None of the other methods succeed even to this extent.

The experiments purporting to prove that the positive Wassermann reaction of syphilitic serum is due to a labile physicochemical change in the serum colloids, readily duplicated *in vitro*, have therefore not been confirmed.

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STUDIES IN THE SEROLOGY OF SYPHILIS

VIII A NEW FLOCCULATION TEST FOR THE SERUM DIAGNOSIS OF SYPHILIS*

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WITHIN the last ten years there has been an increasingly widespread use of various precipitation tests for syphilis. Their simplicity contrasts sharply with the laborious complexity of the Wassermann reaction, and although their value as compared with that of the Wassermann is still controversial, they undoubtedly represent a very significant advance in the serologic diagnosis of syphilis.

It is perhaps unfortunate that so many of these tests have been devised. In principle they are all identical, and only superficial variations in technique distinguish most of them. In the face of the recognized value of the Kahn test, the Meinicke-Klarungs-reaction, the Muller-Ballungs-reaction, and several others it would seem useless to add still another test to an already confusing array were it not that its sensitivity, technical simplicity and ease of reading all made possible by the use of a new sensitizing substance are difficult to reproduce in any of the tests now available.

I THE USE OF CORN GERM STEROL AS A SENSITIZING SUBSTANCE

In the precipitation tests for syphilis the "antigen" is an alcoholic extract of animal tissue, usually, beef heart. When diluted with saline, this extract forms a milky suspension of lipid particles which remain stable when added to normal serum, but agglutinate in syphilitic serum to form visible aggregates.

It is known that the addition of cholesterol to the alcoholic tissue extract gives rise to a more opaque fluid when the extract is diluted with salt solution, this increased opacity being due to the formation of a larger number of microscopically visible particles. Elsewhere I have brought forward evidence to support the view that these particles consist of a core of cholesterol covered with a superficial film of lipid which constitutes the reacting surface. The larger particles so formed are, for obvious reasons easier to agglutinate into visible clumps. Moreover such "complex" lipid cholesterol particles have for an unknown reason a greater tendency to combine with reagin, explaining the greater efficiency of the cholesterolized antigen in the Wassermann reaction also †.

According to the foregoing conception the sensitizing action of cholesterol is due solely to its physical properties. If this is true one might predict that any substance with similar physical properties would have a similar action,

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furthermore, since the degree of sensitization increases directly with the amount of cholesterol, it might be predicted that the discovery of such substances would allow the preparation of much more sensitive antigens for both the Wassermann and precipitation tests than have hitherto been available

These expectations have been realized in the discovery of a group of alcohol-soluble, water-soluble substances which, on addition to the alcoholic tissue extract, increase its sensitivity in exactly the same manner as does cholesterol. The use of one of these, sitosterol, to produce a more sensitive Wassermann antigen has been described in detail elsewhere*. Since the publication of that paper, another sterol derived from corn germ has been obtained through the cooperation of the Difco laboratories. The physical properties of this substance make it particularly fitted for use in a precipitation test.

When a cholesterolized antigen† is diluted with approximately two volumes of saline, it forms a highly unstable suspension consisting of coarse visible aggregates of particles. The evidence outlined in the first paper of this series indicated that the elements of these aggregates are tiny amorphous particles of cholesterol covered by the active lipid. If one adds corn germ sterol instead of cholesterol to the beef heart extract, and dilutes as before with two volumes of saline, one obtains a somewhat more stable suspension, microscopically similar to that formed by a cholesterolized antigen. When this suspension is examined microscopically, however, its opacity is seen to be due to myriads of tiny needle crystals, instead of the amorphous particles formed by a cholesterolized antigen. When a small portion of the suspension is added to normal human serum, the crystals remain discrete, and the mixture appears homogenous and diffusely opaque. When shaken, the cloud of refractile discrete crystals is readily visible. In syphilitic serum, however, the crystals rapidly aggregate to form coarse clumps.

II PREPARATION OF THE ANTIGEN

For the basic alcoholic extract, dry powdered beef heart (100 grams) is extracted three times with four volumes of pure anesthesia ether (400 cc) at 37° C, each extraction lasting ten minutes with frequent shaking. This serves to remove unstable substances (fats, soaps, sterols, etc). After the third filtration,‡ the moist powder is washed on the filter paper with one volume of ether (100 cc), dried thoroughly, and extracted for three days with 5 volumes of 95 per cent ethyl alcohol. The ether extracts are discarded. The alcohol extract is filtered, and the moist powder washed with fresh alcohol until the washings are colorless. The combined alcoholic filtrate and washings (about 650 cc) are then evaporated on the steam bath to a volume corresponding to 5 cc per gram of original powder (500 cc). The lipid content of the final extract is usually around 1.6 per cent. Six-tenths per cent cholesterol and 0.6 per cent corn germ sterol are then added and dissolved by boiling to form the stock antigen. Kept in the icebox, it is good for at least twelve months.

*J. Exp. Med. 53: 605 1931

This antigen is used in a 1:200 dilution prepared by dropping the antigen slowly with shaking into 200 volumes of 0.85% NaCl. This has been found to be a more satisfactory method of dilution than that recommended in the paper cited.

†Tissue lipoids 1½ per cent cholesterol 0.6 per cent

‡With suction to prevent undue evaporation

III TECHNIC OF THE TEST*

The antigen is heated at 65° to 85° C for a few minutes to dissolve the excess sterol, and 13 volumes of 4 per cent NaCl rapidly blown into 1 volume of the antigen. This antigen dilution should be allowed to "ripen" for at least half an hour. Unlike the Kahn antigen, this dilution need not be used within a certain short period of time. If kept in the icebox it can be used as long as 3-6 days after its preparation. Because its sensitivity gradually increases as it ages, the routine procedure in this laboratory is to prepare the dilution one day in advance allowing it to ripen in the icebox for twenty-four hours before use.

The serum to be tested is inactivated for twenty to thirty minutes at 56° C, and 1/8-1/12 its volume of the antigen dilution added. If the antigen suspension is older than three days the amount added to the serum should be decreased to 1/15 the serum volume. Although the test can be carried out with 0.2 cc of serum and 0.02 cc of antigen dilution, it is more convenient to use twice these quantities. The turbid mixture is then shaken for two minutes. The incubation period can be adjusted at will to suit the circumstances.

If a rapid reading is necessary, as for an emergency transfusion, the tube is incubated at 37° C for one-half hour. Otherwise, it may be incubated at 37° C for 4 to 8 hours. It need hardly be added that the longer the incubation period, the more sensitive are the results obtained. Our routine procedure is to incubate for 4 hours at 37° C. In any event, after incubation, the tube is centrifuged at 1500 r.p.m. for 10 to 15 minutes. Three volumes of NaCl N/7 (0.85 per cent) are then added, i.e., three times the volume of serum used.

In a negative reaction the tube is seen to be homogeneous and diffusely opalescent. On shaking one sees a cloud of tiny refractile crystals not visible if the tube is at rest. In a positive reaction these crystals clump to form coherent coarse white floccules floating in a clear and transparent fluid. There is the sharpest possible contrast between the water-clear fluid and coarse floccules of the positive test, and the homogeneous opacity of the negative test.

One occasionally encounters weak positives, particularly in patients under antisyphilitic treatment. Usually, aggregation in such cases is definite, but even when there is only a slight granular appearance, resembling a positive Kahn, not sufficiently marked to justify a definite reading of *positive*, a second centrifugation at higher speed usually enables one to evaluate the results in terms of positive or negative. If aggregates are really present, they are thrown down to form a coherent floccule at the bottom of the tube, covered by a clear supernatant fluid. In a negative, the crystals remain discrete and are not thrown down; the tube remains homogeneous and opalescent.

Although the macroscopic reading is far more satisfactory, it is possible to read the results by microscopic examination. In a negative test one sees myriads of tiny crystals which do not cohere even though they are in immediate contact. In a positive test these crystals are clumped in much the same manner that red cells are clumped by an agglutinating serum, leaving clear spaces between the aggregates.

*The principles determining the choice of quantities, conditions, etc. will be discussed in a forthcoming paper dealing with precipitation reactions in general (Am. J. Syph. April 1932).

It is strongly urged that laboratories using the test restrict themselves to the following terms in reporting results *positive*, *negative*, and *doubtful*, the latter calling for a repeat test

IV ADVANTAGES OF THE TEST

The points of superiority of this test as compared with current serologic methods are briefly enumerated below. The statistical evidence on which the first two points are based will be presented in a later paper.

1 *Sensitivity*—The reaction is very sensitive, distinctly superior in this respect both to the immediate Kahn reaction, which is acknowledged to be among the best of the flocculation tests for syphilis, and to a four-hour icebox Wassermann test with a sensitive antigen. It detected >70 per cent of a known syphilitic population under specific treatment. The Kahn reaction on the same group of cases yields about 60 per cent positive reactions. These results are to be compared with those obtained in the 1928 League of Nations Conference, at which the best tests detected around 60 per cent of a known syphilitic population.

2 *Specificity*—The results obtained in testing a general dispensary population indicate that the incidence of false positive reactions is less than $\frac{1}{4}$ per cent, as judged by the history, clinical signs, Wassermann reaction, or a combination of any or all of these criteria. In a control series of 190 medical students, there was not a single positive reaction.

3 *Simplicity of Preparation of Antigen*—This is sufficiently obvious from Section I.

4 *Simplicity of Technique*—Only one tube is necessary, and but 0.2-0.4 c.c. of clear serum. No chemicals are necessary save salt solution, and no apparatus save pipettes, water-baths, and centrifuge.

5 *Ease of Reading*—The results are so clear cut as to reduce to a minimum the factor of interpretation. One need not scrutinize the tube very closely for tiny aggregates, as in the Kahn test, when positive, the flocculation is apparent and requires no trained technician for its reading. Occasional doubtful results can be usually resolved into a definite positive or a definite negative by a second centrifugation at higher speed.

6 *Cheapness*. The most expensive ingredient is the most essential one—the corn germ sterol. Despite the present high cost of this substance, the cost per test is only a fraction of a cent. One c.c. of antigen makes 23 c.c. of the final antigen dilution, sufficient for either 50 or 100 tests, depending upon whether 0.2 or 0.4 c.c. of serum are used.

7 It is suitable for immediate tests, as for an emergency transfusion, in which case results can be read within an hour after the blood has been obtained, as well as for a highly sensitive four-hour or overnight test.

8 The antigen dilution keeps for at least three days if stored in the ice box, indeed, the longer it stands, the more sensitive it becomes. Our routine procedure is to dilute the antigen with saline a day before it is used.

9 The test is not so finely adjusted that the slightest variation in technique will make for false negative or false positive results. Surprisingly large variations in the amount of saline used in diluting the antigen (1.1-2 c.c.), in the

age of the antigen dilution (one-half hour to three days) in the proportion of antigen dilution to serum (1:4-1:16) and in the time of incubation (one-half hour to 24 hours) do not appreciably affect the results obtained

10 Quantitative Test A quantitative test is readily performed, by determining the dilution in which a serum will still give a positive reaction as illustrated in the following set-up

c c serum	0.4	0.2	0.1	0.05					
serum diluted 1:16, c c					0.4	0.2	0.1	0.05	
NaCl N/7	0	0.2	0.3	0.35	0	0.2	0.3	0.35	
Antigen dilution, c c	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	
Reading	+	+	-	-	-	-	±	-	

The serum is positive up to a 1:32 dilution, i e, contains 32 units

SUMMARY

A flocculation test for syphilis is described based upon the use of a beef heart extract sensitized with both cholesterol and corn germ sterol. Dried powdered beef heart (100 g) is extracted three times with four volumes of anesthesia ether, each extraction for ten minutes at 37° C. The dried residue is extracted for three days with five volumes of 95 per cent ethyl alcohol. After filtration, the powder is washed with 95 per cent alcohol and the combined filtrate and washings evaporated down to 500 c c. Cholesterol and corn germ sterol* are then added each to a final concentration of 0.6 per cent (600 mg for 100 c c of antigen).

A measured volume of the finished antigen is diluted with 1:3 volumes of 4 per cent NaCl. After ripening for at least half an hour (preferably overnight) 0.04 c c of the suspension is added to 0.4 c c of inactivated serum (i e, previously heated at 56° C for thirty minutes) the tube shaken for two minutes, and incubated at 37° C for four hours. It is then centrifuged at >1500 r p m for ten to fifteen minutes. 1:2 c c of 0.85 per cent NaCl are added and results read.

The reaction is sensitive to a degree (>70 per cent of known syphilitics) highly specific (<1/4 per cent false positives) and simple to carry out. The results are clear-cut: a negative serum appearing homogeneous and diffusely opalescent and a positive serum yielding a clear fluid with coarse coherent floccules. The several points of advantage of this test are enumerated in the text. Statistical results in more than 25,000 tests will appear in a following paper.

It should be pointed out that the test as described in this paper is not definitive. The discovery of other sensitizing substances which can be used to replace or supplement cholesterol and corn germ sterol may well make for an increased sensitivity and clarity without endangering the specificity of the results.

*Prepared by Digestive Ferments Co. Detroit.

LABORATORY METHODS

THE VERNES-BRICQ-YVON PHOTOMETER*

ITS APPLICATION TO ROUTINE BIOCHEMICAL WORK, WITH SPECIAL REFERENCE TO
THE ESTIMATION OF PHOSPHORUS IN BLOOD

E OBERMER, M D, AND R MILTON, B Sc LONDON, W 1, ENGLAND

THE serologic application of the Vernes Bricq Yvon Photometer and its use for the turbidimetric standardization of vaccines, have given rise to a considerable literature. Very little has been published however on its application to chemical analysis. We have not been able to trace any references in British or American literature. For references to serologic and vaccine work in the English language Adelaide Bayliss¹ and Wansey Bayly et al² can be consulted. For a full description of the apparatus with illustrations, details of serologic technique, and a brief chapter on its application to chemical analysis with microchemical technique, the reader is referred to Leger and Martin³.

Our own experience with the instrument has convinced us that it possesses overwhelming advantages for routine biochemical analysis. We now use it exclusively in place of the usual colorimeters and nephelometers.

The photometer is an extremely sensitive instrument for the measurement of color or turbidity in definite mathematical terms. The introduction into the eyepiece of a monochromatic screen renders matching a simple process and does away with the usual colorimetric error due to optical idiosyncrasies of the individual observer. The devisors of the instrument claim accuracy to within three scale divisions. In this laboratory an experience of two years has shown all our technicians capable of measurements which agree within one division of the scale. In applying the instrument we have found that, for a general concentration of turbidity or color, the ratio of light transmitted to light absorbed, viz, optical density, is constant, providing the conditions of preparation of the solutions are identical. For a further discussion of these conditions, the book referred to above³ will be found useful.

For the past six years we have been engaged in elaborating methods for the routine analysis of all normal constituents of blood, urine, and feces. The scale of work has necessitated the reexamination of present methods with the object of standardizing technique in such a way as to satisfy the following criteria

- 1 The use of minimum amounts of biologic material
- 2 Simplicity of technique
- 3 Saving of time and bench space
- 4 Accuracy

We first tested out the photometer for the analysis of blood urea⁴. Up to this time we had been using a microgravimetric xanthidol method.⁵ This was

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extremely accurate but necessitated much time and the use of a Sartorius micro-balance. A long series of experiments over a six-month period checked by a macrogravimetric method, showed that the photometric method furnished an even higher degree of accuracy than the gravimetric method. The photometric method was also extremely rapid and simple and necessitated the use of only 0.2 cc serum.

We then proceeded to try out the instrument for various other routine analyses. We now have two Vernes-Bricq-Yvon photometers in constant use in the laboratory. The following routine analyses are carried out exclusively by photometric methods:

Phosphorus fractions in blood
 Phosphorus in urine and feces
 Sulphur fractions in blood and urine
 Magnesium in blood, urine and feces
 Uric acid in blood and urine
 Urea nitrogen in blood
 Amino nitrogen in blood
 Cholesterol in blood
 Hemoglobin in blood
 Creatine and creatinine in urine
 Protein in urine
 Calcium in urine

We are working at present on the standardization of further micro-photometric methods for the estimations of

Sodium in blood, urine and feces
 Potassium in blood, urine and feces
 Chlorine in urine
 Sulphur in feces

Of the above methods only the photometric blood urea technic has been published as mentioned above. Recently a microphotometric method for the estimation of blood sulphur fractions has been published.⁶ The technic independently worked out by these authors is very similar to our own. Our present technic for the routine analysis of the other constituents of blood, urine and feces will be published in book form in the near future. In view however of the fact that this instrument has been little or not at all used in the routine biochemical laboratory, we have considered it opportune at this juncture to call attention to its advantages. As an illustration we propose to give the details of a micro-photometric system for the analysis of the blood phosphorus fractions. We have also considered it useful to record the experimental work leading up to the choice of our present technic.

METHODS FOR BLOOD PHOSPHORUS ESTIMATION

Previously we have had at our disposal a choice of methods based on the following principles:

- 1 Volumetric
- 2 Gravimetric
- 3 Nephelometric
- 4 Colorimetric

For routine estimation of the various phosphorus fractions in blood, volumetric and gravimetric methods were found unsuitable. They necessitated the use of too large quantities of blood. We were left with present micromethods which can be divided into two groups

- 1 Nephelometric or turbidimetric
- 2 Colorimetric

TURBIDIMETRIC METHODS FOR PHOSPHORUS ESTIMATION

Existing Methods—The literature contains several accurate methods based upon the capacity of phosphorus for combining to form a white insoluble strychnine phosphomolybdate

- (a) Embden⁷ first utilized this principle in his gravimetric technique
- (b) Myrback⁸ and Roche⁹ precipitated the strychnine salt in nitric acid solution and used a titrimetric endpoint
- (c) Rona and Kleinmann¹⁰ have published the only present method for turbidimetric estimation of phosphorus

For straightforward phosphorus estimation, Kleinmann's method (as published by Rona and Kleinmann), is to be recommended. The author however specifies that the solution containing phosphorus must be devoid of all organic matter before precipitation can be attempted. This necessitates washing in the case of blood or trichloroacetic acid filtrate, and is therefore not suitable for inorganic phosphorus estimation.

Attempted Photometric Application of Turbidimetric Methods—Of the above, the only principle which lent itself to a possible photometric application, was that of Myrback and Roche. We give details of our technique below.

Five tenths cc clear plasma are added to 1 cc water. This is mixed with 1 cc of 20 per cent trichloroacetic acid. After standing the precipitate is filtered off through a phosphorus free filter. Five tenths cc of the clear filtrate is transferred to a small hemolysis tube. Two cc of water and 1 cc of strychnine nitromolybdate reagent are added. This latter is prepared extemporally by mixing 3 cc of a solution of 16.5 per cent ammonium molybdate with 33 per cent strong nitric acid to 1 cc of a 1 per cent strychnine solution.

The contents of the hemolysis tube are allowed to stand for twenty minutes. At the end of this period the tube is inverted several times in order to obtain homogeneity. The optical density is then read in the photometer. This optical density is compared on a previously constructed graph relating optical density to concentration.

Experimental Results Using Photometric Application of Turbidimetric Principles—Using a standard solution of KH_2PO_4 , the figures arrived at are shown in Table I.

These figures show that a constant turbidity, i. e., constant variations of optical density, can be arrived at when a pure phosphorus solution is used.

TABLE I

MG P IN SOL	OPTICAL DENSITIES		
0.001	9	10	9
0.002	23	25	23
0.003	33	35	35
0.004	50	54	50
0.005	66	64	66
0.006	85	87	85
0.007	101	100	101
0.008	118	117	117
0.009	135	135	135
0.010	148	152	150

Before applying this method to blood, we endeavored to ascertain the influence of trichloroacetic acid on the stability of suspension. Using the proportions of free and neutralized trichloroacetic acid demanded by the technique, arrived at the figures shown in Table II.

TABLE II

OPTICAL DENSITIES			
MG P IN SOL	PURE SOL	SOL + 0.25 C C 20 PER CENT TRICHLOROACETIC ACID	SOL - 0.25 C C SODIUM TRICHLOROACETATE
001	10	7	10
003	35	23	26
005	65	49	57
007	100	97	91
009	135	145	120

These figures show that the addition of the reagent alone interferes with stability of suspension to such an extent as to make the method unreliable. A concomitant series of experiments using blood provided confirmatory evidence. As a standard of comparison we used the orthodox Briggs Colorimetric method mentioned below. This had previously been used as a routine method in this laboratory (Table III).

TABLE III

INORGANIC PHOSPHORUS CONTENT OF PLASMA			
BRIGGS COLORIMETRIC METHOD		PHOTOMETRIC APPLICATION OF ADOPTED TURBIDIMETRIC PRINCIPLE	
4.05 mg	% P	3.94 mg	% P
4.10 mg	% P	3.15 mg	% P
4.10 mg	% P	3.40 mg	% P

In view of these findings, the question of suspension stability seemed too complex to warrant further work along these lines. We decided therefore to confine ourselves to a colorimetric technique.

MICROCOLORIMETRIC METHODS FOR PHOSPHORUS ESTIMATION

Existing Methods.—The literature contains a large number of accurate microcolorimetric methods. The more important of these can be tabulated as follows:

- a Bell and Doisy¹¹ first suggested the use of molybdic acid in order to produce a blue color.
- b Briggs¹² modified the above using hydroquinone bisulphite.
- c Benedict and Theis¹³ incorporated minor improvements in technique.
- d Kay and Robinson¹⁴
- e Maitland and Robison¹⁵
- f Stanford and Wheatley¹⁶
- g Deniges¹⁷ put forward the principle of the reduction of phosphomolybdic acid with stannous chloride.
- h Kuttner and Cohen¹⁸ carefully investigated Deniges' principle, and showed that definite limits of reacting substances gave specific reduction for phosphorus although both arsenic and silica might be present in the solution in large amounts.
- i Youngburg and Youngburg¹⁹ confirmed this work and elaborated a system for the microanalyses of the various phosphorus fractions in blood.

- j Fiske and Subarrow²⁰ made very extensive investigations on the possible substances which will reduce phosphomolybdic acid. They selected aminonaphthol sulphonic acid 124 for phosphorus reduction in the cold, and found that this substance gave a more intense blue color than all others tried by them.

Photometric Applications of Colorimetric Technique—On theoretical grounds we considered the following three methods out of the list given previously, most suitable for investigation: (1) Fiske and Subarrow,²⁰ (2) Benedict and Theis,¹² (3) Youngburg and Youngburg.¹⁹

Attempted Photometric Application of Fiske and Subarrow Method—Photometric application of this principle was found impracticable owing to the extreme instability of the aminonaphthol sulphonic acid reagent. The reducing strength of this solution apparently varies from hour to hour. While this fact does not impair its utility for colorimetric purposes, it would necessitate preparing a fresh graph for each photometric estimation. This method was therefore abandoned as unsuitable.

Experimental Results Using Photometric Application of Benedict and Theis's Colorimetric Technique—The following technique was adhered to: To 1 cc clear plasma add 2 cc of 20 per cent trichloroacetic acid and 2 cc of distilled water. Shake and stand for five minutes. Filter through a phosphorus free filter paper. To 2 cc of the filtrate add 5 cc of water, 1 cc of hydroquinone bisulphite solution, and 1 cc of molybdic acid solution. Place in a boiling water bath for ten minutes. Cool under running water and read in the photometer.

In applying this technique we find that particular care must be taken to ensure constant conditions. Benedict and Theis recommend fifteen minutes boiling. The figures given in Table IV indicate that this period is too long. The greenish tinge which appears after ten minutes depresses optical density. We ensured standard conditions by the following means:

TABLE IV

P CONT. OF SOL.	TIME OF BOILING	OPTICAL DENSITY
0.020 mg	5 min	35
	7 min	45
	9 min	65
	11 min	64
	13 min	60
	15 min	57
	17 min	57
	19 min	50
		} Greenish tinge

The tubes were placed in a boiling water bath adjusted so that the contents reached boiling within one minute.

At the end of ten minutes the tube was taken out and cooled to room temperature under running water. If the tube is allowed to cool spontaneously, a considerable loss of time is involved, and the chances of error are greater. Oxidation is apt to occur at the top of the tube even if stoppered.

A graph relating optical density to concentration was prepared from a standard solution of KH_2PO_4 , as shown in Table V.

TABLE V

AMT. OF P IN SOL.	EQUIV. % OF P USING 2 CC OF FILTRATE	OPTICAL DENSITIES		
0.005 mg	1.25 mg	16	17	17
0.010 mg	2.50 mg	34	34	34
0.015 mg	3.75 mg	52	52	52
0.020 mg	5.00 mg	68	69	68
0.025 mg	6.25 mg	89	85	86
0.030 mg	7.50 mg	105	106	106
0.035 mg	8.75 mg	126	125	125
0.040 mg	10.00 mg	143	144	143

Using a sample of human blood plasma, the method was tried out against the original colorimetric technic, and the following figures were arrived at

PHOTOMETRIC METHOD

420 mg % P
425 mg % P
430 mg % P
425 mg % P

COLORIMETRIC METHOD

40 mg % P
425 mg % P
410 mg % P
420 mg % P

A long series of parallel estimations showed that it was possible to get good results with the above technic. On the other hand the method necessitated extreme care with the boiling conditions and other manipulative details. The method was also a lengthy one. All these factors decided us against adopting it for routine purposes.

Experimental Results Using Photometric Application of Youngburg and Youngburg's Method—We next attempted a photometric application of Youngburg and Youngburg's method. The theoretical advantages were

- 1 Small amount of blood required
- 2 Stability and purity of reagents, none of which are organic
- 3 Specificity for phosphorus
- 4 Saving of time in manipulation

First we proceeded to apply the original technic as given by Youngburg and Youngburg with the following exception. For inorganic phosphorus we used 1 cc of filtrate representing 0.2 cc of plasma, instead of 2 cc as in the original technic. For total acid soluble phosphorus we took 1 cc of blood filtrate, instead of 2 cc as in the original technic. A graph was prepared using a standard solution relating optical density to concentration as shown in Table VI.

TABLE VI

ACTUAL P IN SOL.	EQUIV % P	OPTICAL DENSITY
0.002 mg	1 mg	16
0.004 mg	2 mg	31
0.006 mg	3 mg	45
0.008 mg	4 mg	60
0.010 mg	5 mg	74
0.012 mg	6 mg	88
0.014 mg	7 mg	103
0.016 mg	8 mg	117
0.018 mg	9 mg	131
0.020 mg	10 mg	144

Before proceeding to carry out estimations of phosphorus in blood, we made a series of experiments to test the accuracy of the method.

Effect of Substances in Physiologic Quantities on Color Produced—Table VII shows the results arrived at when substances usually met with in blood plasma were added in physiologic or somewhat larger quantities.

TABLE VII

SUBSTANCE ADDED	AMOUNT OF PHOSPHORUS FOUND				
	MG	MG	MG	MG	MG
Nil	0.020	0.016	0.012	0.008	0.004
0.5 cc 20% Trichloroacetic acid	0.0199	0.0158	0.0120	0.0082	0.0041
0.2 cc 2% Urea	0.0196	0.0160	0.0118	0.0078	0.0040
0.2 cc 1% NaCl	0.020	0.0158	0.0120	0.0082	0.0040
0.2 cc 1% MgCl	0.0202	0.0156	0.0120	0.0084	0.0040
0.2 cc 1% CaCl	0.0206	0.0158	0.0120	0.0080	0.0038
0.2 cc 0.1% Uric Acid	0.0204	0.0160	0.0120	0.0078	0.0038
0.2 cc 2% Glucose	0.020	0.0160	0.0120	0.0084	0.0038
0.2 cc 0.5% K ₂ SO ₄	0.0206	0.0166	0.0120	0.0076	0.0034

It will be seen that the color produced is not affected by substances even well over physiologic limits. The small differences are no greater than could be attributed to experimental error, with one exception. It will be noticed that sulphates do have a definite although small effect. This point will be taken up again when we consider the method as applied to the analysis of total acid soluble and lipid fractions in blood.

Effect of Varying Amounts of Stannous Chloride—Kuttner and Cohen¹ state that the limits of concentration of stannous chloride for color development are 0.020 and 0.022 per cent stannous chloride in the final solution. Kuttner and Lichtenstein¹ on the basis of further work state that this is too conservative and that the optimal zone of color production can be extended to 0.01-0.03 per cent. The figures in Table VIII tend to confirm that the original limits as given by Kuttner and Cohen are too narrow. It will be seen that very little variation in color is produced between such wide limits as 0.015-0.03 per cent.

TABLE VIII

P CONT	OPTICAL DENSITY WITH VARYING CONCS OF STANNOUS CHLORIDE					
	0.005% SnCl	0.01% SnCl	0.015% SnCl	0.02% SnCl	0.03% SnCl	0.04% SnCl
0.004 mg	34	32	31	31	29	34
0.008 mg	65	62	59	60	58	67
0.012 mg	103	95	90	88	85	90
0.016 mg	142	129	120	117	118	124
0.020 mg	170	152	146	144	142	148
Nil	14	15	20	20	26	27

Satisfied with these preliminary experiments we proceeded to test out the method on "routine bloods" in the laboratory.

Experimental Results Using Blood Plasma—1. *Estimation of Inorganic Phosphorus Fraction*. Youngburg and Youngburg's original technique was adhered to. The reader will find details of the technique as modified for photometric use, at the end of this article. Estimations in triplicate on two bloods gave the following typical results:

a	3.4	3.35	3.35 mg per cent Phosphorus
b	3.80	3.80	3.80 mg per cent Phosphorus

In order to test the accuracy of these figures we carried out the following experiments:

1. Parallel estimations in comparison with the photometric application of Benedict and Thies's method (Table IX).

TABLE IX

NAME	BENEDICT'S ADAPT	YOUNGBURG & YOUNGBURG ADAPT
MM	4.8 mg % P	4.7 mg % P
FA	5.2 mg % P	5.15 mg % P
FV	4.7 mg % P	4.5 mg % P
MM (serum)	4.6 mg % P	4.5 mg % P
	4.6 mg % P	4.6 mg % P
	4.8 mg % P	4.6 mg % P
	4.5 mg % P	4.7 mg % P
F	4.6 mg % P	4.8 mg % P
	5.2 mg % P	4.6 mg % P
O'Br	5.0 mg % P	5.1 mg % P
	3.35 mg % P	3.6 mg % P
H	4.6 mg % P	4.7 mg % P
	4.6 mg % P	4.7 mg % P
	4.6 mg % P	4.6 mg % P
	4.8 mg % P	4.6 mg % P
XCIV		

2. Parallel estimations with a micromethod using large amounts of animal blood (Table X).

TABLE X

MICRO ESTIMATION (0.2 CC PLASMA USED)	MACRO ESTIMATION (120 CC PLASMA USED)
3.5 mg per cent	3.30 mg per cent
3.5 mg per cent	3.30 mg per cent
3.6 mg per cent	3.50 mg per cent
3.5 mg per cent	3.46 mg per cent
3.5 mg per cent	3.46 mg per cent

(The macromethod was a titrimetric application of Neuman's method of precipitation of ammonia phosphomolybdate, the retural technic being an application of the method given by Rona and Kleinmann¹ for phosphorus in urine)

3 Recovery of known quantities of phosphorus added to blood plasma (Table XI)

TABLE XI

ORIG P CONT OF BLOOD	PHOS ADDED	PHOS FOUND	PHOS RECOVERED	% PHOS RECOVERED
0.0071 mg P	0.002 mg	0.0092 mg	0.0021 mg	105
	0.002 mg	0.0092 mg	0.0021 mg	105
	0.004 mg	0.0111 mg	0.0040 mg	100
	0.004 mg	0.0113 mg	0.0042 mg	105
	0.006 mg	0.0132 mg	0.0061 mg	102
	0.006 mg	0.0132 mg	0.0061 mg	102
0.0041 mg P	0.002 mg	0.0061 mg	0.0020 mg	100
	0.004 mg	0.0081 mg	0.0040 mg	100
	0.006 mg	0.0100 mg	0.0059 mg	98.4
	0.008 mg	0.0121 mg	0.0080 mg	100
	0.012 mg	0.0160 mg	0.0119 mg	99.7

B Estimation of Acid Soluble and Lipoid Fractions When we proceeded to apply the technic, with the slight quantitative modifications mentioned above, to the analysis of the acid soluble and lipid phosphorus fractions, we found difficulties during the process of oxidation, which we shall go into in some detail.

Youngburg and Youngburg discussed the use of H_2O_2 as an oxidizer, first introduced for phosphorus estimation by Brumann.² We agree in finding it far superior to the other oxidizers for this purpose. Even here the excess of H_2O_2 must be removed. A green color is produced with the molybdic acid solution if the slightest trace is present, and the development of a blue color on addition of stannous chloride is inhibited. We were therefore careful to chase out the final traces of H_2O_2 by following the flame from the bottom to the top of the tube several times. When during the course of these experiments we were obliged to make up new quantities of molybdic acid, we noticed that results were not concordant with previous findings. On titration we found that the acid content of the "10/N" acid from a different Winchester was slightly deviated. With reference to this subject, Kuttner and Cohen³ state that the limits of sulphuric acid in the final solution should be between 0.9/N and 1.05/N. In our experience this range is confirmed for specificity of the method for phosphorus. We were however, unable to agree that the color development with an equal amount of phosphorus in the solution, is equal for 0.90 and 1.05/N respectively. We found that extraordinarily small deviations in the normality of the acid had a definite effect on the optical density of the color produced. This factor also agreed with the definite effect on color production of adding sulphates to an ordinary solution, as mentioned in Table VII. Thus we found it necessary to plot out a fresh graph for the standard phosphorus solution whenever a new batch of reagents were prepared. From the point of view of photometric technic, a graph is of use only when the amount of sulphuric acid in the unknown solution is rigidly identical with that which was

present in the graph solution. Following on from this we were led to go into the whole question of oxidation. When the original technic of Youngburg and Youngburg is adhered to, it is a fact that some H_2SO_4 is used in the oxidation process, and that some is also lost in the sweeping out of the final traces of H_2O from the combustion tube.

Using great care to avoid unnecessary loss of H_2SO_4 , we carried out experiments to assess the degree of error introduced by loss of acid.

Using 3 cc of plasma divided into three equal parts, we prepared three trichloroacetic acid filtrates in the usual way. One and five tenths cubic centimeters of each filtrate was used, and heated with 0.75 10/N sulphuric acid and oxidized with H_2O . The solution was then boiled, cooled and completed to 15 cc. Thus 15 cc was divided into three equal parts for the purposes of the experiment. (In fact only 4 cc could be used for one part, owing to unavoidable pipette error.) The three portions of 15 cc each were utilized as follows:

- 1 Twenty five hundredths of a cubic centimeter of 10/N H_2SO_4 was added to the 5 cc and the analysis completed exactly as per Youngburg and Youngburg's modified technic, as adapted to photometric use. The amount of phosphorus found in each of the three specimens is

0.00422 mg

0.00407 mg

0.00407 mg

- 2 Five cubic centimeters was titrated to find the sulphuric acid content. We found [0.25 cc 10/N H_2SO_4 gave an equivalent figure of 2.54 cc of N NaOH]

1 5 cc of solution contained 2.36 cc N H_2SO_4

Loss = 0.18 N H_2SO_4

2 5 cc of solution contained 2.38 cc N H_2SO_4

Loss = 0.16 N H_2SO_4

3 5 cc of solution contained 2.4 cc N H_2SO_4

Loss = 0.14 N H_2SO_4

- 3 Four cubic centimeters was corrected for lost sulphuric acid before completing the estimation, and our findings were as follows:

[As the volume of solution was 4 cc instead of 5 cc, it was necessary to add 0.3 cc 10/N H_2SO_4 to the 4 cc]

SUPPLEMENTARY H_2SO_4 ADDED	PHOS. CONT.	CORRECTED FOR 5 cc
1 1.44 cc	0.00316	= 0.00395 mg
2 1.28 cc	0.00316	= 0.00395 mg
3 1.12 cc	0.00316	= 0.00395 mg

A similar series of experiments were carried out on the Lipoid fractions. Three portions of the same plasma were precipitated and extracted in 10 cc alcohol ether. Six cubic centimeters of each filtrate was oxidized with 1 cc 10/N H_2SO_4 . After boiling the oxidate was completed to 15 cc.

This solution was utilized as follows:

- 1 Five cubic centimeters of the solution was estimated exactly as per the Youngburg and Youngburg modified technic as adapted to photometric use. We found

1 Phosph. contained = 0.01020 mg

2 Phosph. contained = 0.00990 mg

3 Phosph. contained = 0.01005 mg

- 2 Four cubic centimeters of solution was titrated to estimate acid lost in oxidation. [0.5 cc 10/N acid gave 5.08 cc N/1 NaOH]

1 3.47 cc of N NaOH reqd i.e. 4.34 cc for 5 cc

Loss = 0.74 cc N

2 3.56 cc of N NaOH reqd i.e. 4.45 cc for 5 cc

Loss = 0.63 cc N

3 3.54 cc of N NaOH reqd i.e. 4.42 cc for 5 cc

Loss = 0.66 cc N

3 Five cubic centimeters of the solution were corrected for lost acid and the phosphorus content ascertained. We found

SUPPLEMENTARY ACID ADDED		PHOS CONT
1	0.74 cc	0.00944
2	0.63 cc	0.00944
3	0.66 cc	0.00924

These results are summarized in Table XII

TABLE XII

ACID SOLUBLE FRACTION			LIPOID FRACTION		
DIRECT ESTM	MODIFICATION ADDING LOST ACID	% ERROR	DIRECT ESTM	MODIFICATION ADDING LOST ACID	% ERROR
0.00422 mg	0.00395 mg P	6.9%	0.01020 mg	0.00944 mg P	7.5%
0.00406 mg	0.00395 mg P	2.6%	0.00990 mg	0.00944 mg P	4.7%
0.00406 mg	0.00395 mg P	2.6%	0.1005 mg	0.00924 mg P	7.6%

These findings show a percentage error at least sufficiently great to be taken into consideration. This would in our experience also apply to the original Youngburg and Youngburg colorimetric technic. We suggest therefore, that workers using this technic should take cognizance of this source of error and correct for it.

To summarize our conclusions derived from the experimental results with Youngburg and Youngburg's technic described above, we finally came to the conclusion that the original Youngburg and Youngburg technic is suitable for photometric adaptation to the analysis of inorganic phosphorus. It is not suitable for the estimation of total acid soluble and lipid fractions without modification. If, however, any acid lost in the oxidation process is collected, it gives excellent results.

DETAILED DESCRIPTION OF PHOTOMETRIC TECHNIC FINALLY ADOPTED

For the use of workers who intend to use the photometer for routine analysis of phosphorus fractions in blood, we append the exact technic used in this laboratory. For the establishment of the preliminary graph using a standard KH_2PO_4 solution, we refer readers to Table V. We desire also to call attention to the necessity of using clear plasma (or serum), which must be free from any traces of hemolysis. The routine followed in our laboratory is to use a portion of the plasma collected under paraffin for the estimation of blood alkaline reserve; this is rapidly separated off from the corpuscles by centrifugalization.

Reagents—1 Sodium Molybdate solution

112 gm molybdic acid in 450 cc water. Neutralize with caustic soda (about 54 gm). Boil for thirty minutes and make up to 200 cc.

2 Sulphuric Acid

37.5 cc sulphuric acid added to 100 cc water.

3 Molybdic Acid Solution No. 1

50 cc 7.5 sodium molybdate plus 50 cc 10% sulphuric acid.

4 Molybdic Acid Solution No. 2

50 cc 7.5 sodium molybdate plus 50 cc 5% sulphuric acid.

5 Stannous Chloride

10 gm stannous chloride in 25 cc conc hydrochloric acid. Dilute 1 cc to 200 cc with water for use.

6 Alcohol Ether

75 cc alcohol mixed with 25 cc ether.

7 Twenty per cent Trichloroacetic acid

8 Hydrogen peroxide (40 vols)

9 N/1 H_2SO_4 solution (must be exact)

10 N/1 NaOH solution (must be exact)

Technic—Preparation of Filtrate To 0.8 cc plasma add 1.6 cc water, mix in a test tube. Then add 1.6 cc 20 per cent trichloroacetic acid and shake vigorously. Allow the mixture to stand for fifteen minutes, filter.

1 *Inorganic Phosphorus Fraction* Take 1 cc of the filtrate, 6 cc water, and 2 cc molybdic acid solution No. 1. Mix, add quickly 1 cc of the dilute stannous chloride solution. Allow to stand for about five minutes, read the depth of color produced in the photometer.

Prepare a control tube, and replace the filtrate by distilled water. Note the difference in these two readings on the special graph giving the result in mg phosphorus per 100 cc blood.

2 *Total Acid Soluble Phosphorus Fraction* Take 1.2 cc of the protein free filtrate. Add 0.6 cc of 10/N sulphuric acid in a Pyrex tube. Heat with addition of hydrogen peroxide until a clear solution is obtained, taking care (a) to avoid unnecessary loss of sulphuric acid, and (b) to insure sweeping out excess of hydrogen peroxide in the tube. Cool, add 2 cc water and boil. Cool, transfer quantitatively to a 10 cc graduated cylinder. Complete to 6 cc and mix.

Pipette out 1 cc of the solution into a small flask. Titrate with N/1 soda from a microburette. Calculate the amount of sulphuric acid in the remaining 5 cc. Do a control titration at the same time on 0.5 cc of 10/N sulphuric acid, thus calculating the amount of sulphuric acid lost during combustion. Add the amount of sulphuric acid lost using NH_4SO_4 to the 5 cc in the cylinder, complete to 7 cc with water, add 2 cc molybdic acid solution No. 2 and 1 cc stannous chloride. Read in the photometer after five minutes.

3 *Lipid Phosphorus Fraction* Pipette 3 cc of the ether alcohol mixture into a test tube with a 4 cc graduation mark, using a fine bore pipette add 0.2 cc plasma slowly, cork and shake well, bring to boil, stand for ten minutes, cool, complete to 4 cc, shake to mix, and filter. Then 2.4 cc of filtrate is evaporated to dryness in a hard glass test tube. Six tenths of a cubic centimeter of 10/N sulphuric acid is added, and the contents cautiously heated and oxidized with hydrogen peroxide. The subsequent estimation of phosphorus is as for acid soluble phosphorus, care being taken to make good any acid lost in combustion.

SUMMARY

1 It is claimed that the Veines-Blaug-Yvon photometer represents an advance over all other colorimeters and nephelometers commonly used.

2 Reasons are given for this claim. A table is given showing the chemical constituents of blood, urine and feces for the analysis of which photometric methods have been evolved in this laboratory. As an illustration of the steps taken to evolve rapid photometric methods for routine laboratory use, the estimation of phosphorus fractions in blood is discussed in detail.

3 Existing nephelometric methods for blood phosphorus estimation are discussed. The photometric application of these methods is impractical. Reasons are given for this statement.

4 Existing microcolorimetric methods for blood phosphorus estimation are discussed.

5 Experiments are discussed showing the attempted application of the ammoniaphthol sulphonic acid 1.2.4 phosphorus reduction method. Reasons are given for its rejection.

6 A description is given of an attempted photometric application of Benedict and Theis's modification of Briggs colorimetric method. It is found to give accurate figures. Its disadvantages are given as reasons for not adopting it as a routine laboratory method.

7 A photometric application of Youngburg and Youngburg's colorimetric method is described.

8 Experiments are described proving the accuracy of this method for the estimation of the inorganic phosphorus fraction in blood

9 Experiments are described showing the source of error in the oxidation process of Youngburg and Youngburg's technique. This renders Youngburg and Youngburg's technique accurate for the analysis of the total acid soluble and lipid phosphorus fractions in blood

10 A modification is suggested which does away with this source of error

11 A microphotometric technique for the analysis of the phosphorus fractions in blood is given in detail. This method has been used for some time as a routine in the authors' laboratory, and found to be simple, time-saving and extremely accurate

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PRELIMINARY REPORT ON THE USE OF AN IMPROVED FORM OF ELECTROCARDIOGRAPH*

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THE introduction by Einthoven of the string galvanometer, or electrocardiograph, in 1903, and its subsequent use in the laboratory and in the clinic marked an important advance in the understanding of certain cardiac conditions

There are now available for electrocardiography many models of the string galvanometer type and a few combining a vacuum tube amplifier with an oscillograph. Doubt has been cast upon the accuracy of the latter type of instrument.¹ Ernstene and Levine² obtained records with both instruments and found only small differences in the records, they concluded that the amplifier-oscillograph type of electrocardiograph gave records of sufficient accuracy to warrant continuation of its use, basing this conclusion upon the assumption that the string galvanometer was a suitable standard of comparison. This assumption however, in view of the results discussed below, is not tenable. Actually, the results obtained from the two types should be different if the amplifier-oscillograph equipment is properly designed.

DEFECTS OF THE STRING GALVANOMETER

It would seem that the medical profession has assumed that the string galvanometer is a suitable instrument, in the sense that it will faithfully record the variations in electrical potential that occur in the human heart. However, this opinion is not concurred in by electrical engineers, they use the oscillograph almost exclusively for recording such variations in electric potential.

A detailed discussion of the defects of the string galvanometer is about to be published by members of the Department of Electrical Engineering of the Massachusetts Institute of Technology, I am permitted to abstract some of this article.³ Two significant defects are discussed. First it is shown that the wave forms recorded by the string galvanometer become distorted when frequencies much in excess of 20 cycles per second are present. In addition a wave (the QRS from a normal electrocardiogram) was subjected to what is known as harmonic analysis, a form of mathematical analysis, and it was disclosed that the frequency of the components involved greatly exceeded 20 cycles per second. It is stated³ "The relative amplitudes show that these higher harmonics are of great importance in determining the wave form, and any failure to respond equally at both high and low frequencies must result in distortion of the wave."

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The second source of error in the recording of changes of electrical potential, i.e., in the electrocardiogram, by the string galvanometer lies in the presence of body resistance in the measuring circuit. To quote³ "Under average conditions, the resistance of the human body is about 2500 ohms between terminals such as are used in electrocardiographic studies. The galvanometer resistance is usually of the same order of magnitude. Hence the current which flows in the galvanometer is controlled as much by the internal resistance of the body as by the external resistance of the measuring circuit. Although the galvanometer resistance is substantially constant, the body resistance may vary and thus alter the shape of the wave on the record."

USE OF AN IMPROVED FORM OF ELECTROCARDIOGRAPH

An apparatus is described³ which more suitably fulfills the principle requirements of any electrocardiograph, which are first, it must produce records which accurately depict the variations of electromotive force impressed upon it, second, the records thus produced should be of sufficient size to permit accurate interpretation. It consists principally of a special tube amplifier and an oscillograph.

In the following is presented a preliminary report of the application of this new instrument in the field of electrocardiography, and a few records obtained by its use.

Approximately 70 tracings have been taken of 56 patients, on both the string galvanometer and the new equipment. An analysis of the voltage recorded, or

TABLE I

COMPARISON OF VOLTAGE RECORDED BY AMPLIFIER TYPE AND BY IMPROVED FORM OF ELECTROCARDIOGRAPH WITH THAT RECORDED BY AN EINTHOVEN STRING GALVANOMETER, TWO SERIES OF 25 PATIENTS IN EACH COMPARISON.

WAVE	STATUS ¹	NUMBER OF LEADS ²		AVERAGE DIFFERENCE		MAXIMAL DIFFERENCE ³	
		AMP ²	IMP ²	AMP ²	IMP ²	AMP ²	IMP ²
P	same	23	19				
	lower	48	15	0.02	0.02	0.05	0.07
	higher	4	41	0.01	0.04	0.01	0.18
Q	same	49	37				
	lower	21	12	0.03	0.02	0.05	0.06
	higher	5	26	0.02	0.03	0.02	0.13
R	same	10	3				
	lower	64	24	0.10	0.09	0.37	0.34
	higher	1	48	0.08	0.19	0.08	0.90
S	same	28	34				
	lower	43	31	0.05	0.09	0.20	0.34
	higher	4	10	0.05	0.04	0.10	0.11
T	same	32	23				
	lower	41	12	0.05	0.03	0.11	0.05
	higher	2	40	0.01	0.03	0.01	0.15

¹ As compared with same wave recorded by string galvanometer.

² The amplifier type instrument tested by Ernstone and Levine.

³ The improved form of electrocardiograph reported in this article.

⁴ Standard three leads for each patient, so number of leads total 75.

⁵ In millivolts.

the amount of the excursion from the baseline, shows that it is not identical in the records obtained by the two instruments. The details are given in Table I, in which is included a summary of the results obtained by Ernstene and Levine² in their comparison of the electrocardiograms taken by the Einthoven string galvanometer and an amplifier-type electrocardiograph. Their comparison was based upon a series of twenty-five cases, as is ours in Table I. The fact that the

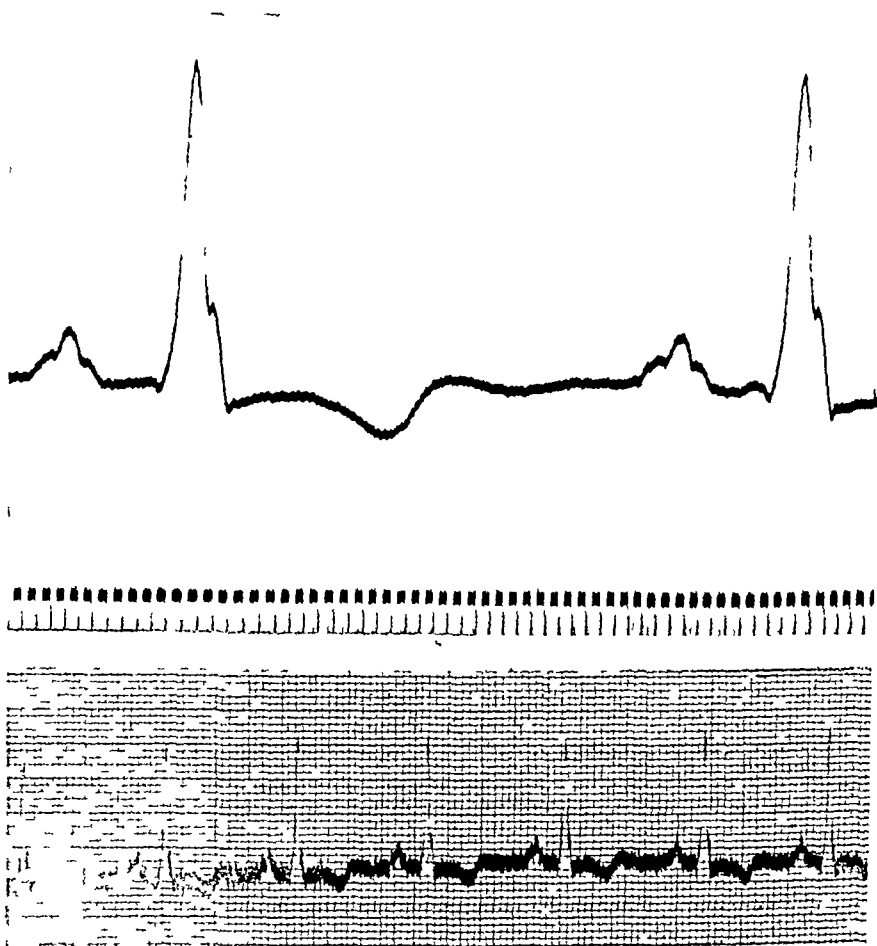


Fig 1—Normal rhythm from case of rheumatic mitral stenosis. Note difference in P-waves and R-waves. In this and in the subsequent illustrations the new electrocardiogram is mounted above that taken by the string galvanometer type is below. The time marker indicating one fiftieth of a second is at the bottom of new record. In the string galvanometer record the time intervals are indicated by the conventional abscissae: the fine ones equal one-twenty-fifth of a second; the coarse ones equal one-fifth of a second.

standard three leads were recorded in each of the 25 patients causes the figures given to total 75.

In brief, Table I presents a check, in amount of electrical potential recorded in millivolts, of the new apparatus against the string galvanometer and against the amplifier type of electrocardiograph tested by Ernstene and Levine². Subject to the limitation that the patients used by the latter and by me were not

the same individuals the suggestion is that their amplifier type of electrocardiograph and the new apparatus do not give identical results

The latter equipment permits the spreading out of the electrocardiogram, if desired to such a degree that but a single cardiac beat is recorded on a 36-inch strip of paper. The waves can be amplified without loss of accuracy, to almost the full width of standard oscillograph paper ($3\frac{3}{4}$ inches). In the present study,

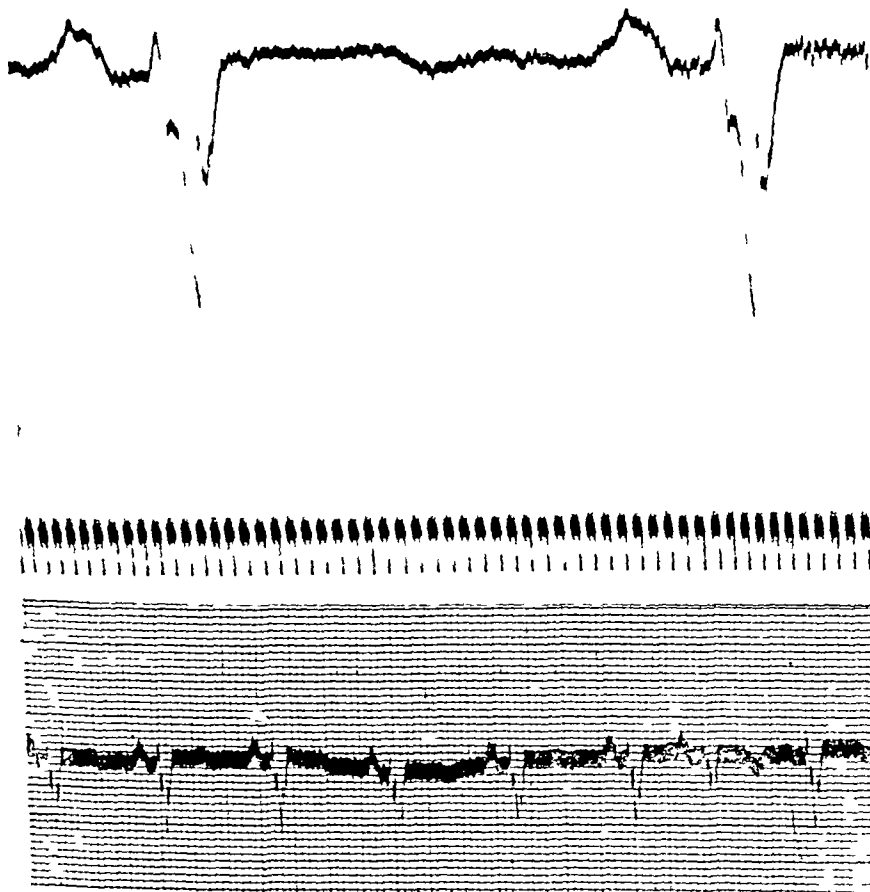


Fig 2—Normal rhythm. Note details of the QRS complex

however, attention has been concentrated upon a practical (Figs 1 to 6) rather than upon the freak size

Some of the oscillations in Figs 1 to 6 do not originate in the heart of the patient. Since these electrocardiograms were obtained, further investigation has been made by Caldwell Oler and Peters; they discuss³ the sources of these fine oscillations and methods for their prevention.

DISCUSSION

There is a desire to utilize changes in detail of the electrocardiogram in the diagnosis of impairment of the myocardium such as is prone to occur in the

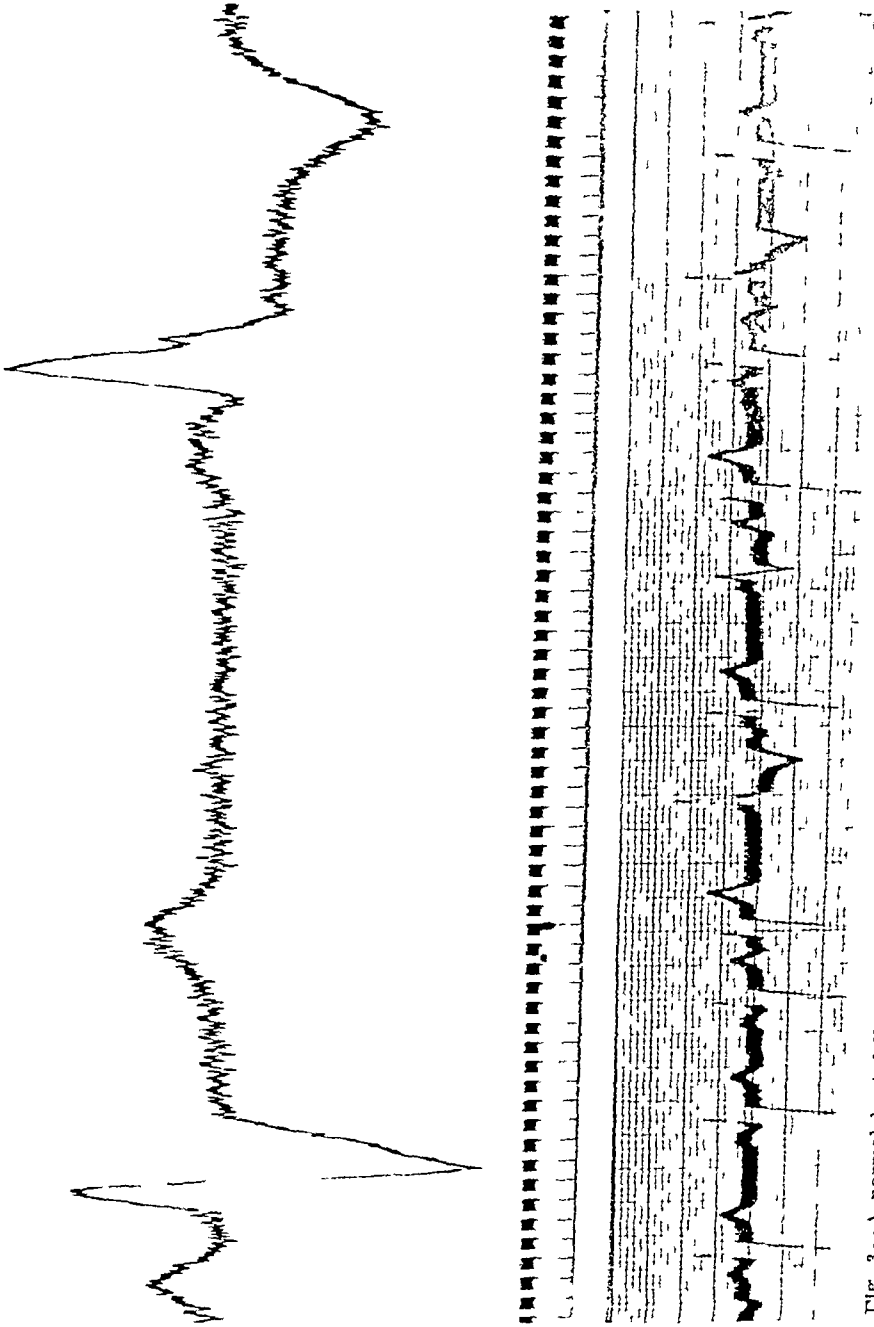


Fig 3—\ normal beat followed by an extrasystole of ventricular origin. A considerable number of the fine oscillations mentioned in the text occur in this record

presence of coronary thrombosis. There can be little dispute that if small differences are to be of diagnostic value, the electrical record must be accurate. Confidence in the accuracy of the electrocardiogram cannot, in view of the work of Caldwell et al.,³ be placed in records made by a string galvanometer, it would seem that it can if such or similar equipment as they describe be used.

Some waves are of insufficient amplitude and many notchings are too inconspicuous to be readily studied in electrocardiograms taken according to the present standardization on the instruments commonly used. This improved form of

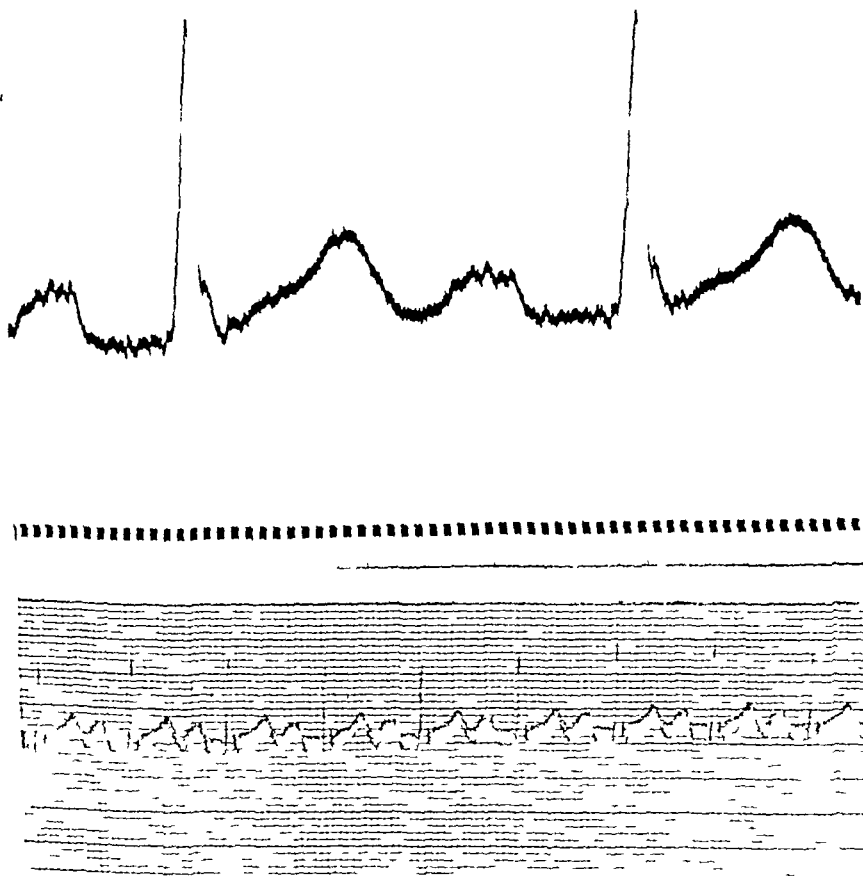


Fig 4—Delayed A-V conduction. The P-P interval measures 0.27 second.

electrocardiograph makes it possible to amplify and spread out the electrocardiograms without loss of accuracy. One accustomed to the study of electrocardiograms experiences the sensation that now he can study under high power, of the microscope, records which formerly could be viewed only under low power. Reference to Figs 1 to 6 will disclose the manner in which the improved equipment records the barely visible notchings and thickenings of waves in the conventional type of electrocardiogram as definite notchings and alterations in contour of the respective waves.

It is distinctly probable that the standardization that is part of the conven-

tional technic of electrocardiography makes the record too small to properly disclose some of its details. Adoption of a technic that produces a somewhat larger and more spread out electrocardiogram, facilitated by this improved equipment, gives promise of disclosing new information pertaining to cardiac physiology. Examination of the electrocardiograms already obtained with this improved equipment gives me the temerity to advocate such a change in the standardization employed (provided the improved equipment, or similar is used) in electrocardiography.

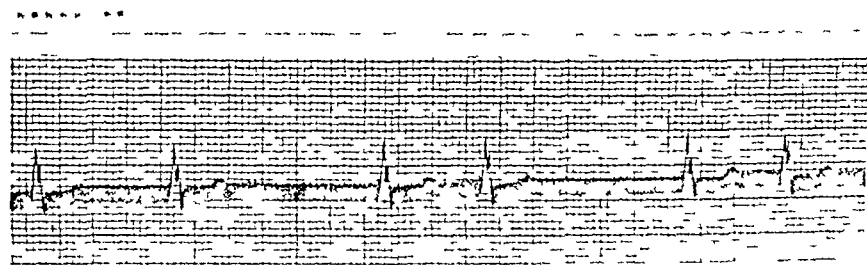


Fig. 5—Auricular fibrillation. Some difference is apparent in the contour of the QRS complex.

It is appreciated that during recent years at least three electrocardiograph equipments using vacuum tube amplifiers with an oscillograph have appeared upon the market. These produce records similar in appearance to those made by the Einthoven string galvanometers. They undoubtedly possess some of the advantages of the equipment used in the instrument described by Caldwell, Oler, and Peters, but as the latter state: "No attempt is made to improve upon the conventional type of record." It is emphasized that the workers at the Massachusetts Institute of Technology did not seek to reproduce the record obtained by the string galvanometer, but directed their efforts towards assembling an equipment that would produce the best type of record of the changes in electrical potential occurring in the human heart.

It is not possible as yet to state what will result from the use of this improved form of electrocardiograph in the field of electrocardiography. It may

take years with some laboratory experiments and with many of these new electrocardiograms correlated with the clinical records and necropsy findings, to determine what new information, if any, can be obtained by the use of this more accurate and more efficient instrument. There can be little doubt that the opportunity afforded by the new equipment thoroughly warrants full and hopeful investigation.

There are numerous problems of research along physiologic lines which need an instrument more capable of recording minute changes in electrical potential.*

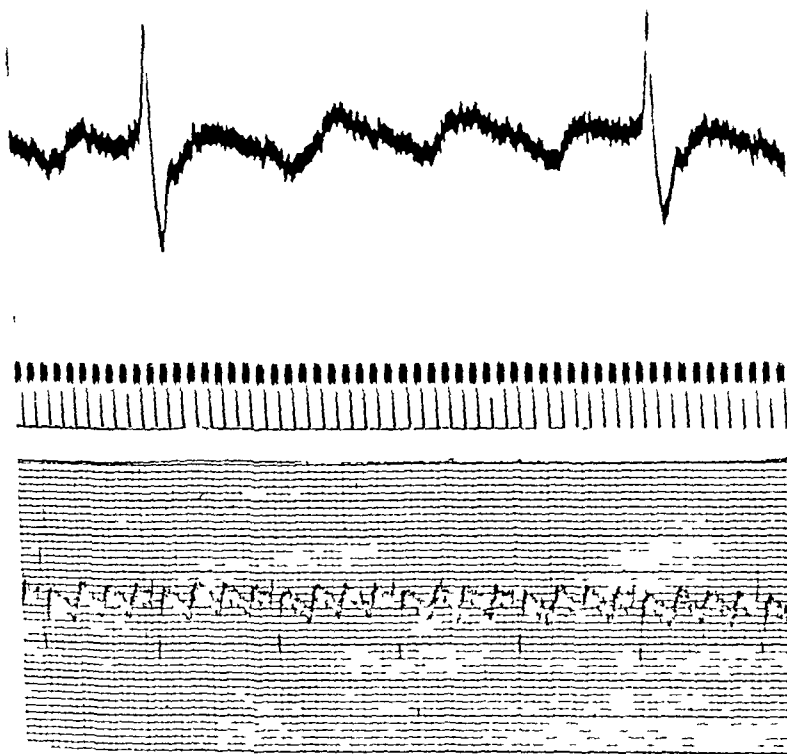


Fig 6—Auricular flutter

It is believed that this improved form of electrocardiograph represents a suitable instrument.

This preliminary report is offered particularly for the information of those who desire to avail themselves of the opportunities afforded by a better instrument for electrocardiography and physiologic research.

SUMMARY

The Einthoven string galvanometer is not a suitable instrument for electrocardiography in the sense that it does not faithfully record the rapid variations in electrical potential that take place in the heart.

*F. H. Pratt, Professor of Physiology at Boston University School of Medicine, already has recorded with this apparatus curves produced by the extremely low action potential of the anterior and posterior lymph hearts in the frog.

The improved form of electrocardiograph is suitable for such work. The workers at the Massachusetts Institute of Technology directed their investigation to the assembling of an equipment that would faithfully record the rapid changes of electrical potential occurring in the heart, and that would produce the best form of record, rather than of attempting to reproduce the conventional electrocardiogram.

This improved equipment permits the amplification and spreading out of the electrocardiogram, without loss of accuracy, so that the details of its waves may be studied. Adoption of a technic that produces a somewhat larger and more spread out record is advocated.

It seems hopeful that the future use of this improved form of electrocardiograph will disclose new information pertaining to the heart.

This improved equipment should also be of definite assistance in the pursuit of problems in physiology requiring the recording of rapid changes in electrical potential.

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ANEROID-TYPE OF TAMBOUR FOR RECORDING RESPIRATORY MOVEMENTS AND INTRATHORACIC PRESSURE*

BURGESS GORDON, M.D., PHILADELPHIA

CERTAIN difficulties are experienced with the rubber diaphragm type of tambour in recording changes in respiration. The tension of the diaphragm varies and the thread which secures the rubber to the bell of the instrument may become loose. As a result fallacies may occur in the tracing and not infrequently it is necessary to adjust the apparatus during the experiment. Since the tension of the rubber may vary, comparative studies with the same "set up" are not entirely correct. Furthermore the rubber may disintegrate due to heat, the action of foreign substances and when not in use. To replace the rubber is time-consuming.

The following is a description of an instrument which has been found useful in recording changes in the intrathoracic pressure, the rate and depth of respirations in man and animals. A housing 7.5 cm. in length and 3.5 cm. in width (divided into two compartments) contains aneroid and eccentric units. The aneroid consists of five chambers which are constructed from "crimped" German silver sheeting .025 mm. in thickness. A valve stem with an opening into each chamber passes through the center of the aneroid and into the outer

*From the medical service of Dr. Thomas McCrie and the Department for Diseases of the Chest, Jefferson Hospital.
Received for publication December 8, 1931.

wall of the housing where it is connected with brass tubing 14 cm in length. This tubing serves as the conduit for air and is the part for attachment of the tambour to the upright stand. The distal end is connected with the rubber tubing which leads to the pneumothorax needle or chest recording hose. The other end of the valve fits into a collar which is screwed to a shaft 0.5 cm in length. This passes through the partition and into the other compartment where it is connected by means of a pinion to the lateral arm of the eccentric. The central part of the eccentric is clamped to a concave movable base which

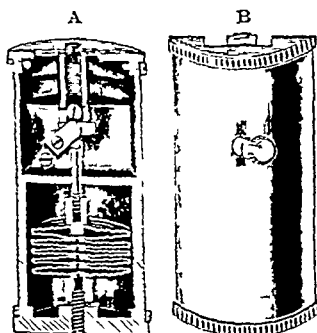


Fig 1

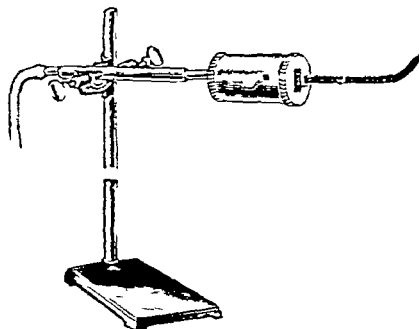


Fig 2

Fig 1—A. This is a cross section showing the aneroide and eccentric units. L shows the set screw which regulates the swing of the eccentric and recorder.

Fig 2—Shows the tambour attached to the upright stand and a section of the rubber tubing for connection with the pneumothorax needle or chest recording hose.

fits closely in the lateral section of the compartment. The aluminum recorder is clamped in a box which projects from the central part of the eccentric. A partial rotation of the eccentric converts the horizontal motions of the aneroide into vertical movements. The arm and recorder have free excursion through an opening 2.5 cm in width at the end of the chamber. The base of the eccentric is adjustable by means of a set screw. This provides a means for regulating the degree of recorder excursion above or below the neutral line.

The apparatus is light in weight, easily portable, and comparatively rugged in construction.

The instrument is manufactured by Geo. P. Pilling and Son Company, Philadelphia.

A SIMPLIFIED INSTRUMENT FOR MEASURING METABOLISM*

BY ALLEN D. GARRISON, PH.D., HOUSTON, TEXAS

IT HAS occurred to the author that, with the growing demand for measurements of basal metabolism, the instruments available for the purpose present some decided disadvantages from the standpoint of many physicians and technicians who would like to be prepared to make such measurements.

The first disadvantage is one of cost. It would appear that many physicians, particularly those in small towns or otherwise isolated from well equipped laboratories and hospitals, would frequently have occasion to measure basal metabolism, but hesitate to invest \$200 to \$700 in one of the instruments now available.

Furthermore, one familiar with metabolism measurements will also recognize inconveniences other than first cost in instruments which record oxygen absorption rates by a kymograph record, such as that used in the Benedict-Roth or the McKesson designs, have the disadvantage of certain inaccuracies due to irregular breathing, and the resultant serious difficulty of estimating the average slope of a line drawn along the base of a second very wobbly curve, the demand for pure oxygen often amounts to an inconvenience, since tanks must often be shipped from a distance, and while the Douglas, Bailey and similar methods avoid both of these troubles, one is required to be a fairly good gas chemist with considerable capital.

The apparatus illustrated in Fig. 1 has been designed to eliminate, as far as possible, some part of the inconveniences and inaccuracies of metabolism measurements and, at the same time, to fill a need which may not be completely filled by more complicated and expensive instruments, namely, to supply a simple, durable, portable, yet inexpensive and accurate device.

The principle of the measurement is a departure from previous practice. This instrument collects exhaled air over a measured time (a definite number of exhalations), and measures the entire amount of carbon dioxide exhaled in that time. By the use of the respiratory quotient, it is a simple matter to calculate as if oxygen had been measured directly.¹

(A) is a rigid container for the exhaled air which is led into the space (B) from the subject through an ordinary mouthpiece and flexible tubing, containing flutter valves (not shown in the illustration). Exhaled air coming through the flexible tubing (V) and the tube (M) may be directed either into the space (B) of the container (A), or it may be deflected out into the outside air by turning the four-way valve (K).

A diaphragm (D), operating on the principle of a gasometer, divides the container into compartments (B) and (C), and can be operated by pushing or pulling the rod (E) which is equipped with a handle at its upper end and

*From the Department of Physical Chemistry, The Rice Institute.
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emerges through the top of the container through a packing joint (*F*) with a knurled nut for adjusting the compression on the packing. It will be evident that the gasometer diaphragm (*D*) controls the relative volumes of the spaces (*B*) and (*C*) and that the water in the lower half of the container serves as a gas tight seal between these spaces, whether the diaphragm is in the raised or lowered position. The volume of water necessary to effect this seal is reduced by constructing the bottom of the container in the manner illustrated, thereby reducing the weight of the apparatus.

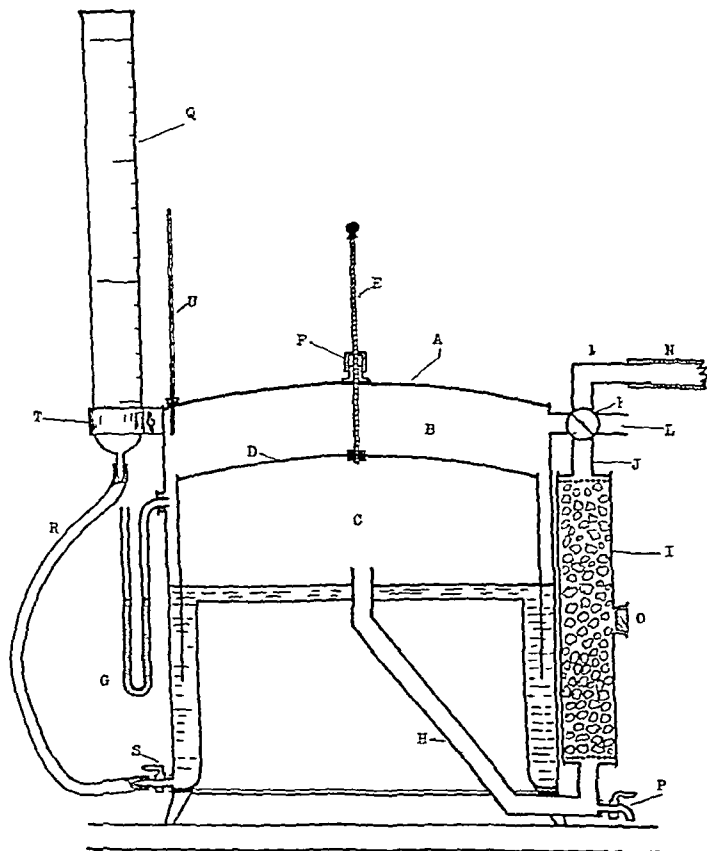


Fig 1

A glass manometer of conventional design is provided at (*G*) to make it possible to see when the pressure inside the apparatus is identical with atmospheric.

The compartment (*C*) is open through tube (*H*) to a container for soda lime or other absorbent of carbon dioxide and thence through tube (*J*) to the four-way valve (*K*). The soda lime container (*I*) is provided with screens at each end and an opening with a suitable gas-tight stopper at (*O*). The cock at (*P*) is provided to drain the bottom of the tube (*H*) of any water spilled from (*C*) or of any liquid alkali from (*I*) and to facilitate the adjustment to atmospheric pressure subsequently described.

A graduated glass cylinder (Q) is provided to measure the quantity of water to be added to the container through the rubber tubing (R) and the stop-cock (S). An adjustable clamp (T) holds the graduated cylinder in position. The graduate (Q) is from 300 to 500 c.c. capacity and graduated in not larger than 5 c.c. divisions.

The thermometer (U) indicates the temperature of the gases in the container.

A metabolism measurement may be conducted as follows. The apparatus, properly filled with water and absorbent for carbon dioxide, is placed by the subject. Mouthpiece and nose clip are applied as usual, and the subject draws air from the room or through a tube from the outside air and exhales it through the tubes (N) and (V) and out through (L) with the valve (K) set as indicated. Graduate (Q) is filled with water to the zero mark, stopcock (S) closed, cock (P) opened, the diaphragm (D) raised to its highest position and the packing nut tightened with the fingers until the friction on the rod (E) just prevents the diaphragm from falling under the force of gravity. The contents of the apparatus will be at atmospheric pressure.

The operator watches the breathing of the subject, preferably by observing the motion of the flutter valve, and just at the end of an exhalation, he turns the valve (K) to the position indicated by the dotted line and starts a stopwatch. Exhaled air enters (B) and drives the diaphragm down displacing the air in (C) through the tube (L). The larger the apparatus the greater the accuracy, but the author has found that twenty to thirty breaths are sufficient to get an accuracy comparable with or better than the accuracy of the recording type of instrument.

When the operator sees that the diaphragm has almost reached its lowest point, he again turns the valve (K) back to the original position and stops the stopwatch at the end of an exhalation. Thus a definite number of breaths are accumulated in a measured time. The carbon dioxide exhaled in this measured time may be estimated as follows.

With the valve (K) left in the position indicated by the heavy line, the cock (P) is left open for a few moments while the air is coming to constant temperature. When the cock is closed the contents of the apparatus are left at atmospheric pressure. If the gasometer diaphragm is lifted, the exhaled air in space (B) will be driven to space (C) through the absorbing substance and the carbon dioxide will thus be removed. Several strokes of the diaphragm will insure the complete removal of the carbon dioxide. This removal will lower the pressure, and during the absorption the cock (S) must be opened and water run in to take the place of the carbon dioxide and keep the pressure at atmospheric.

When the absorption is complete, the diaphragm is lowered to the bottom the position at the start of the absorption, and the apparatus is left standing for a few moments while the temperature becomes constant again and while any water vapor which may have been removed by the absorbent is being replaced by evaporation. Water is then run in or out of the graduate (Q) until the manometer indicates that the pressure is atmospheric. With water in the manometer, this adjustment may be made very accurately, and the amount of carbon dioxide read

from the graduate with an error of less than one per cent. The fall of water in the graduated cylinder (Q) is measured in cubic centimeters and is taken as the volume of the carbon dioxide exhaled in the measured time and absorbed in the apparatus at atmospheric pressure. No correction is necessary for water vapor, since the space is saturated at the start and at the close of the determination and leakage is avoided since the contents of the apparatus is never far removed from atmospheric pressure at any time during the determination.

The author has made comparison determinations of basal metabolism using this device and an instrument of well-known make having a kymograph oxygen-recording equipment. The following data were obtained on one comparison run.

TABLE I
DATA OBTAINED OCTOBER 4, 1931

SIMPLIFIED INSTRUMENT		RECORDING INSTRUMENT	
Temperature	= 26°	Temperature	= 26°
Pressure	= 764 mm	Pressure	= 764 mm
CO volume	= 200 cc	O. Rate from Kymo	
Time	= 1 min. 8 sec	graph	= 1.68 liters
CO Rate/hour	= 10.60 liters	For Time	= 8 min.
Using RQ = 0.82 and correcting for		O. Rate/hour	= 12.6 liters
CO in the air the O. rate was			
calculated to be	= 12.77 liters		
Calories/sq. meter	= 34.8	Calories/sq. meter	= 34.3
Difference = 1.45 per cent			

It will be observed that the instrument described gave a value 34.8 calories per square meter where the recording device gave 34.3 calories per meter. On inspection of the kymograph record, it was found that the extent to which the slope of the breathing line could be estimated may have been in error by as much as the difference observed. Furthermore the time required for the recording was eight minutes while the time for the simplified determination was only one minute, eight seconds. Only fifteen breaths were collected yet the accuracy compares favorably with the recording method. The reason for this is obvious the irregularities of breathing were recorded in one instrument, averaged in the other.

This brings up the question of the most convenient size. The author regards a minute and a half as a minimum time for collecting a sample although he has demonstrated that a little over a minute will give results comparable with the recording method. This means a minimum of twenty to twenty-five breaths, and demands a container at least fourteen inches in diameter and thirteen to fifteen inches high. Larger instruments are more accurate and would not be found inconvenient even for those desiring a portable apparatus. It would not be impractical to make the apparatus as large as the desired accuracy demands. Furthermore, the process of measuring the carbon dioxide contained in twenty to thirty breaths (approximately 200 to 500 cc.) to an accuracy of 2 cc. has its advantages over a recording of larger volumes with larger errors in that the subject is subjected to less inconvenience and it is possible to make two determinations and average the results in the time required to make one measurement of equal accuracy by the oxygen recording method.

Another advantage to be derived from the simplified method is in the elimination of the need of pure oxygen. The subject breathes air, and if that is drawn through a tube from outside a window, the carbon dioxide content will be too small to introduce much error even if neglected. However, it is a simple matter to make a blank determination of the carbon dioxide in the air and let the subject breathe from the air of the room. Other advantages are the simplicity of the operations and the lack of complicated parts and adjustments. Enough soda lime may be provided to make fifty to seventy-five determinations without attention. The manipulation is as simple as a freshman chemistry experiment, and the calculations may be made from the ordinary charts for the purpose.

The author is not able to give an accurate estimate of cost at the present time, but the facts that a ten-dollar stopwatch may be substituted for a kymograph recording device, and that the rest of the apparatus is simple in proportion, indicate that the device could be made at a reasonable figure.

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MODIFICATION OF USE OF WRIGHT'S STAIN*

BY F. D. LA ROCHELLE, M.D., SPRINGFIELD, MASS.

UP TO the time of the appearance of Schilling's book "Das Blutbild und Seine Verwertung" in 1929 we were using Jenner's and Wright's stain for blood smears interchangeably with a slight preference for the latter. These had for years given fair results in a number of instances but neither compare with the results obtained with procedures involving the Giemsa stain. On trying out the technique as described by Schilling, and Schleip and Alder "Atlas der Blutkrankheiten" we obtained good results, but found the process an exceedingly delicate one, and we soon discovered that this delicacy was not due to the stains themselves but rather bound closely with the hydrogen ion concentration of the water. The results from Giemsa stain were so good that we endeavored to simplify the technique, and by making a few modifications, we have been able to obtain with great ease smears that are in every way equal to the best results obtained from Giemsa technique. The technique here described aims at reducing the chances of variations in concentration to a minimum.

The microscope, light, filters, stains, glassware, and especially the water must all be brought to an optimum condition, and this of necessity demands time and attention, but the results are well worth the effort.

The glassware must be clean and of the best quality, and unusual precautions should be taken to prevent contamination with acids or alkalis or other chemicals. The purpose of the technique here described is to make these precautions more or less unnecessary, but they should at least be kept in mind, and if good results are not obtained, it must always be remembered that the water is usually the factor responsible.

Naturally smears must be well made, we usually follow the technic described by Schilling although we find slides about as satisfactory as cover-glasses for spreading the blood

As a rule the time necessary to travel from the patient to the laboratory is sufficient for the smear to dry in air, five minutes is a satisfactory time in an ordinary room. Under no circumstances is heat to be used throughout the process, the small advantage gained is more than offset by the danger of damaging the blood

TECHNIC

Once the smear is dry it is placed over a water glass whose margin must describe an exact horizontal plane if the stain is to flow evenly. Wright's stain is then added, 30 drops is the usual quantity, to cover the smear and in two or three minutes an equal quantity of specially prepared water is added.

Preparation of the water is the important step. We formerly used distilled water but now we prefer tap water. To prepare the water a gallon from the tap is drawn into a clean bottle and a smear stained in the usual way to determine the hydrogen ion concentration, our water is too acid and requires about 5 drops of 5 per cent NaOH per gallon. This varies of course and must be adjusted for every source. A few smears are then stained and NaOH added drop by drop until the desired tint is obtained. In some localities it might take less than 5 drops. But the important point to remember is that bad results come from the water. Once the proper hydrogen ion concentration is found other factors such as stains, time, etc., are of secondary importance and excellent smears are obtained with little care and few failures.

The combination of Wright's stain and water is left on for two or three minutes and to this mixture is added 5 to 6 drops of Giemsa's stock solution and mixed rapidly by tilting the glass, as a rule six to eight minutes is the best time to stain but this may be decreased or increased according to circumstances without material changes in the end-result. In our instance this procedure intensifies somewhat the basic stain and this is offset by washing in tap water that is slightly acid. Naturally the use of tap water for washing is a great convenience.

The smear is then dried in air by standing it on end and examined with oil. We have found liquid petrolatum preferable to cedar oil for this purpose.

This technic may appear complicated but really it is not and can be learned by the average student technician in a few hours, and to us has given more uniform and better smears than any technic used previously.

SUMMARY OF TECHNIC

- 1 Cover smear with Wright's stain, about 30 drops, two to three minutes
- 2 Add equal quantity prepared water
- 3 Add 5 to 6 drops Giemsa's stock solution for six to eight minutes, according to shade desired
- 4 Wash with tap water
- 5 Dry in air
- 6 Examine with liquid petrolatum

THE ESTIMATION OF BILIRUBIN IN BLOOD SERUM*

By MARJORIE PICKENS, B A , and L BAUMAN, M D , NEW YORK CITY

THE determination of bilirubin in serum has become a routine practice in the hospital in cases of jaundice. The quantitative colorimetric estimation of van den Bergh depends on the formation of an azo dye through interaction of bilirubin and diazotized sulphanilic acid. The originator at first employed a diazotized solution of bilirubin itself as a standard of comparison. Bilirubin, however, is very expensive, difficult to obtain in pure form, and is readily oxidized to a green pigment when in solution. For these reasons van den Bergh suggested the use of an ether solution of standard ferric thiocyanate which has a color comparable with that of azobilirubin. In our hands this salt has been preferable to the cobalt solutions more recently recommended.

In the literature we were unable to find a comparison of results when both bilirubin and iron standards were used on the same sample of blood. For a considerable time we were doubtful of the accuracy of the method when the substitute was used. The likelihood of evaporation of the volatile solvent while in the colorimeter cup and the difficulty of exactly matching colors seemed real stumbling blocks. Fortunately, after considerable effort, we secured some chemically pure bilirubin. The results which follow were obtained on the same sample of blood serum with the two standards.

RESULTS OBTAINED WHEN USING THE TWO STANDARDS ON THE SAME SAMPLE OF BLOOD

BILIRUBIN mg %	FERRIC THIOCYANATE mg %	BILIRUBIN mg %	FERRIC THIOCYANATE mg %
44	49	134	130
23	32	53	43
27	32	35	33
31	32	139	140
57	42	37	32
39	36	118	111
14	15	129	130
14	15	89	97
28	21	35	32
28	21	118	100
20	21	105	95
155	154	37	34
136	135	62	60
199	213	66	74
39	46	101	109
90	111	46	53
94	109	90	93
145	125	31	29
91	92	55	46
96	88	67	65
52	45	68	69
117	128	113	106
70	76	38	36

*From the Chemistry Laboratory, Department of Surgery, Presbyterian Hospital and Columbia University
Received for publication October 3 1931

The methods employed were those of Greene, Snell, and Walters¹ for the serum, while the bilirubin standard was prepared and treated according to Chiray and Thiébaud²

The results, while not sufficiently accurate for scientific work, may be regarded as acceptable for ordinary clinical purposes

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A DEVICE DESIGNED TO SIMPLIFY THE HANDLING OF CELLOIDIN SECTIONS*

By MORRIS MASS B S AND NATHAN SCHAFER, B A, M D, NEWARK N J

THERE are distinct disadvantages in the present methods of handling celloidin sections With the method in vogue after the section has been cut and placed in 80 per cent alcohol it is carried through the various solutions by means of a glass rod flattened at one end The section curls about the glass rod and in order to free it a small amount of agitation must be produced In so doing, the tissue tears and separates from the celloidin sheet Since agitation is repeated in each of the fluids tearing is an invariable result at some stage

Again when the section is transferred from the eosin into the concentrated alcohols a marked amount of surface tension is set up and this too causes tearing When the section is carried into the clearing fluid the surface tension is still greater, the section is torn still more and in some instances destroyed

Only expert technicians can obtain good results with the present method and often after a certain amount of failures The work of lifting the sections with a glass rod, and of only running through one section at a time makes the process very tedious This is especially true of eye work where serial sections are desired The time needed to run through twenty sections is approximately five hours and constant care must be practiced throughout

We have devised a simple apparatus to overcome the above disadvantages This apparatus is easily constructed and easily handled The materials consist of ordinary copper window mesh lead solder and small numbered tabs, as are used in numbering animals Squares one and one-half inches in length are cut out of the copper mesh the edges of which are strengthened with a thin border of lead solder The small numbered tab is soldered to one corner Two squares are hinged together using either a strand of wire from the mesh or by making a small hinge out of fine tubing and an ordinary pin (Fig 1) A small clip is made out of a piece of flat metal (Fig 1) In order to facilitate handling of a number of these frames a carrier is made utilizing one of the squares and soldering wire to the edges

*From the Clinical and Pathological Laboratories Newark Beth Israel Hospital
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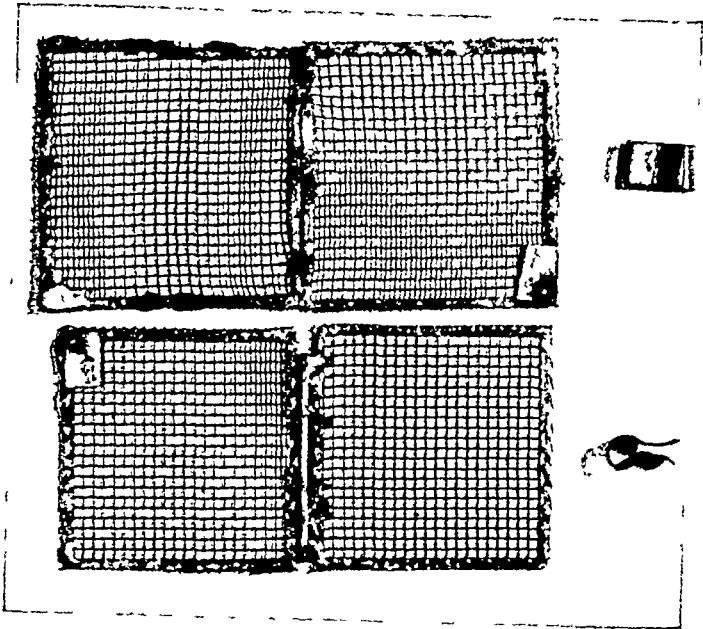


Fig 1

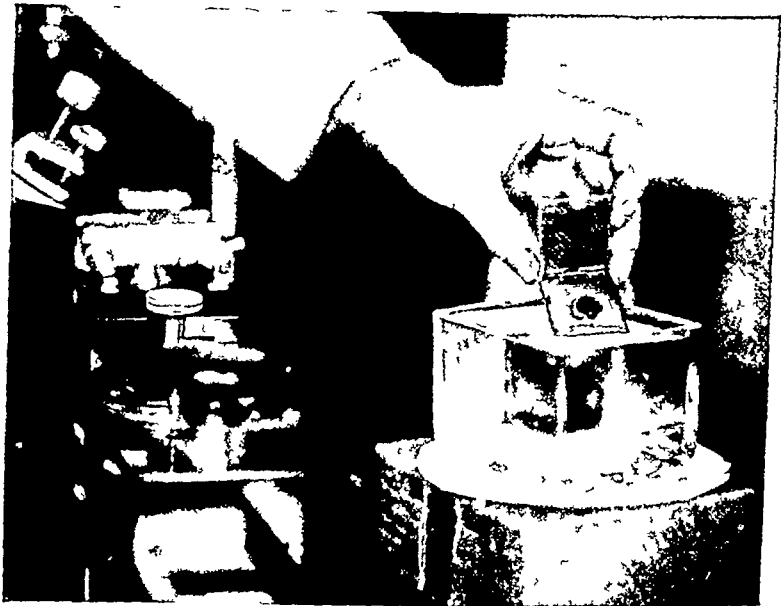


Fig 2

When the section has been cut and placed in 80 per cent alcohol it is floated onto an open frame held between the thumb and first two fingers of the left hand. The section is kept smooth by means of a fine brush held in the right hand (Fig 2). It is then secured by closing the frame and attaching the clip. The closed frame is now placed into the 80 per cent alcohol until needed. There is no danger of any confusion as these frames are serially numbered. The loaded frames are placed on the carrier and dipped into the different solutions. By virtue of their mesh construction the fluid drains easily from the frames and the carrier and the latter may be tapped lightly against the sides of the vessels

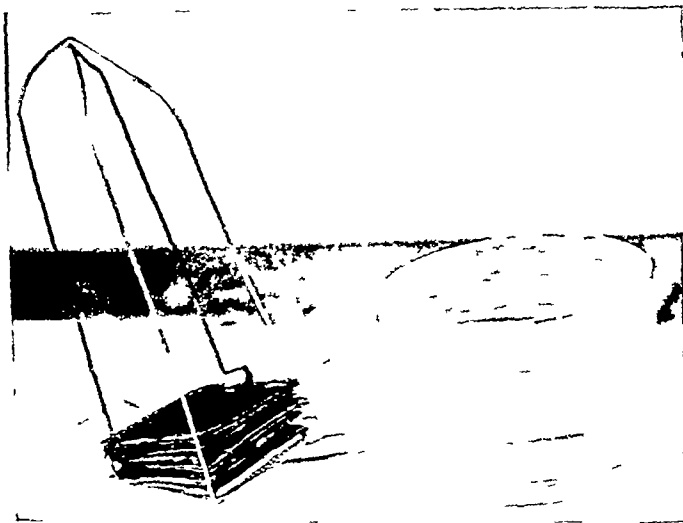


Fig 3

without injury to the tissue (Fig 3). Lastly, the frames are placed into the clearing fluids a few moments, opened, and the sections floated out onto the respectively numbered slides and mounted.

The advantages of this device are readily seen. Manipulation and surface tension are reduced to a minimum. There is little overlapping and wrinkling of the section on the slide. The staining and mounting may be done by an inexperienced technician with no danger of damage to the tissue. The time necessary to run through twenty sections is one-fifth that used in the old method. As many sections may be done at one time as there are frames made. In eye work this device is invaluable, not only because the above disadvantages are eliminated, but the lens is preserved intact.

A CYANHEMATIN STANDARD FOR THE SAHLI HEMOGLOBINOMETER*

By ROBERT D. BARNARD, CHICAGO, ILL.

THE use of pure hemin as a standard for hemoglobinometry has recently been reported by Elvehjem (1931). Such a step may be considered an advance over other methods for preparation of standards, at least in those laboratories where gasometric determinations of oxygen capacity are not available. With pure hemin standards may be weighed out, can be renewed as often as is deemed necessary and form a basis of comparison attainable by widely separated laboratories.

Since 1930, we have been using a standard of cyanhematin prepared from a weighed sample of hemin. Laked hematized blood is diluted with cyanide solution and compared with this standard in the Sahli colorimeter.

SOLUTIONS REQUIRED

1 The standard is made by dissolving 64 mg. of crystalline hemin prepared by the method of Chalfejew† in 1 liter of 1 per cent sodium cyanide solution.

2 A solution of approximately 0.05 molar hydrochloric acid, to which a few drops of caprylic alcohol are added. This solution lyses the blood and converts its hemoglobin to acid hematin. The caprylic alcohol prevents the frothing which is ordinarily troublesome when working with the Sahli colorimeter.

3 A solution of 1 per cent sodium cyanide. This solution is used to dilute the acidified blood in the colorimeter chamber as it converts the acid hematin to cyanhematin.

PROCEDURE

The standard tube of the Sahli instrument is broken off close to the top and the acid hematin suspension is discarded. The tube is then cleaned and filled with the cyanhematin standard (Solution 1). (The original solution prepared in this laboratory has kept its color value for over one year.) The tube may be sealed in a flame or with a small paraffined cork.

For the dilution of the blood sample, the original directions for the use of the Sahli instrument are followed, with the exception that the final dilution is made with cyanide solution. The colorimeter tube is filled to the mark "10" with 0.05 molar hydrochloric acid (Solution 2). Blood is drawn into a 20 mm pipette, the tip is wiped dry, and then placed directly under the level of the hydrochloric acid in the chamber. If this is done carefully, the corpuscles will

*From the Clinical Laboratory of the Chicago Free Dispensary and the Department of Physiology of the Chicago Medical School.

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†500 cc. of defibrinated or citrated blood is added to 2 liters of glacial acetic acid which has been heated to 80° C. As soon as the temperature has fallen to the neighborhood of 55° the liquid is again heated to 80°. The crystals which should form at once are allowed to settle, washed with several changes of distilled water, then with alcohol and finally with ether after which they are dried at 115° C.

form a layer beneath the acid and the pipette walls may be rinsed in the clear supernatant fluid

The contents in the chamber are mixed so that hemolysis occurs. When a deep brown color results 1 per cent sodium cyanide (Solution 3) is added, drop by drop until a color comparison is secured.

COMPARATIVE DETERMINATIONS OF HEMOGLOBIN CONCENTRATION

NEWCOMER METHOD	CYANHEMATIN METHOD
13.75	13.36
13.60	13.54
15.90	15.30
12.30	12.48
12.30	12.17

CALCULATIONS

The value of "100" on the scale of the Sahli instrument with the cyanhematin standard herein described, represents a blood sample which contains 1 millimol of iron or 16.67 gm of hemoglobin in 100 cc of blood. To determine the gram percentage of hemoglobin from the reading of the instrument it is necessary only to divide the latter figure by 6. For example if the color comparison is secured when the meniscus of the fluid in the color chamber is 72, the gram percentage of hemoglobin is $72 \div 6 = 12$. The arbitrary normal of 16.67 is chosen because it represents an even decimal fraction of the molecular equivalent of hemoglobin and is at the same time close to the figure given by Newcomer for the average (16.93) content in human blood.

It is felt that certain disadvantages ordinarily ascribed to the Sahli hematometer have been overcome by adoption of the cyanhematin standard. The advantages of the present method are

1. The simplicity of the original instrument is retained.
2. The colloiddally dispersed hematin, with its complex absorption bands is replaced by a true solution with but one absorption band in the visible spectrum. Theoretically, the latter color will more strictly conform to Beer's law and practically, it is easier to match.
3. The final color value of cyanhematin is attained instantly.
4. It is possible to complete a hemoglobin determination on blood which has inadvertently become clotted in the pipette. Such a sample may be digested with pepsin and hydrochloric acid and then converted to cyanhematin.

REFERENCES

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A TRANSPARENT RULE FOR MEASURING BASAL METABOLISM GRAPHS*

B S LEVINE, PH D, CHICAGO, ILL

THIS paper presents a description of a simple transparent rule for measuring basal metabolism graphs. After two years' experience with this rule, I believe that its advantages are threefold: first, it enables the use of unruled paper with the Sanborn-Giaphic, Sanborn-Benedict and the Roth-Benedict apparatus; second, it can be used in measuring the graphs obtained with any of the machines just mentioned, and third, it gives the final readings directly and simultaneously in terms of cubic centimeters of oxygen consumed per minute and calories of heat radiated per hour. No matter which apparatus is employed, the calculation of the final results is considerably simplified, since the tables compiled for the use with the Roth-Benedict machine can be used with the graphs obtained by either of the two Sanborn apparatus, and vice versa.

The reduced photographic reproduction of the rule shows its simplicity at a glance (Fig. 1).

The relationship between the scale measurements of the rule and the distances of the intersection points, described below, is a trigonometric one, the rule may, therefore, be enlarged or reduced from its original size without in any way disturbing its general applicability. The most suitable size is one of from 8 to 10 inches square.

In using the rule proceed as follows. Place a plain white sheet of paper around the recording drum, securing it by means of small rubber bands. This eliminates the need for pasting and consequent cutting of the recording paper. Prepare the patient and obtain the graphs in duplicate following the usual technique prescribed for the particular apparatus employed. The record should extend over not less than six minutes. Upon the completion of the test period and before removing the drum, make a vertical line with the writing pen by moving it up and down at the middle of the paper, thus dividing the paper into two parts through its entire length, or make an horizontal line by resting the pen in the middle of the paper and turning the drum one complete revolution, thus dividing it into two parts through its entire width. Either of the lines thus made may serve as a base line in the application of the rule.

Remove the graph, place it on a flat surface, and in the manner usually prescribed, draw a straight line through the apices of the respiratory excursions, extending this line along the entire width of the paper.

Apply the rule as follows. Place the rule over the graph so that its vertical or horizontal guide line completely coincides correspondingly with the vertical

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or horizontal base line on the graph. Now, move the rule up or down, along the vertical line or to the right or left along the horizontal line until point of intersection *SG* (if the Sanborn-Graphic is used) or point of intersection *SB* (if the Sanborn-Benedict is used) or point of intersection *RB* (if the Roth-Benedict is used) falls upon the line running along the apices of the respiratory excursions, taking care all the while that the guide line and base line remain absolutely coincident. Note where the respiratory excursion line on the graph intersects the scale line of the rule and read the results by noting the measurement on the right of the scale line, which gives the number of cubic centimeters

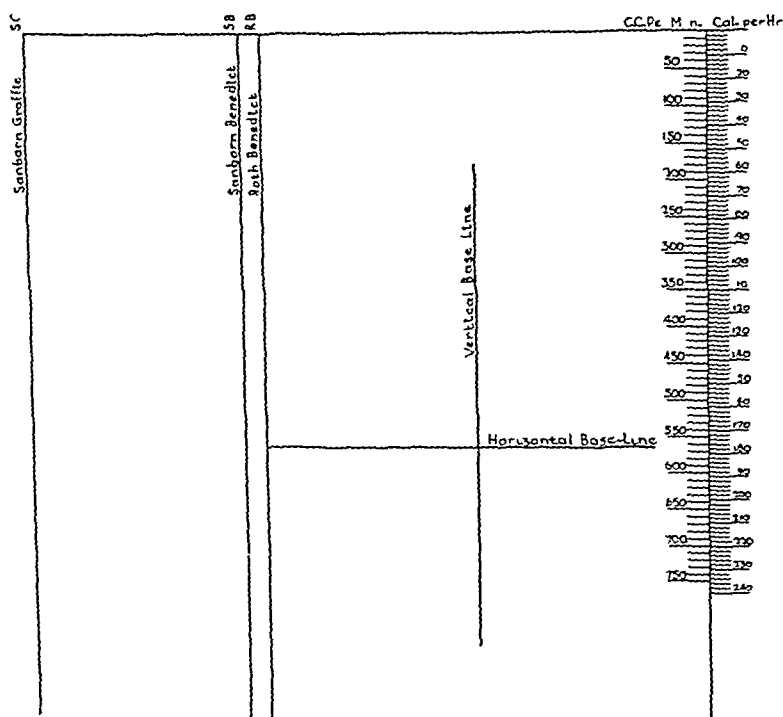


Fig 1

of oxygen consumed per minutes, or the measurement on the left, which gives the number of calories consumed per hour

The final results include corrections for 0° and 760 mm pressure. Corrections for actual temperature, pressure, age, and sex as well as the final percentile calculation, can be accomplished with the aid of the tables given in either the Sanborn or the Collins booklets, or the calculations may be completed by the method of Campbell¹. My experience of many years has indicated to me that in making correction calculations the laboratory technician is more adept with tables than with graphs. By the use of the rule described in this paper and with the aid of either the tables compiled for the Sanborn or Collins apparatus a complete BMR calculation can be carried out in as short a time as one minute.

SUMMARY

A transparent rule is described for measuring basal metabolism graphs which entirely eliminates the need of the expensive heavily ruled paper used heretofore. It shortens the process of calculation by giving final readings in terms of cubic centimeters of oxygen consumed per minute and calories radiated per hour. The rule can be made of translucent paper, celluloid or any other transparent material.

REFERENCE

1. Campbell, Walter R. Nomograms for Metabolism Estimations, J. LAB. & CLIN. MED. 16:1113, 1931.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

ANAPHYLAXIS Prevention of Shock, Waldbott, G. L. J. A. M. A. 98 446, 1932

The following conclusions are drawn from a study of eight fatal cases

1 A negative history of personal or familial allergy or of previous serum injections should not be relied on in ruling out the possibility of serum sensitization

2 Skin or conjunctival tests should be performed before administering serum, but a negative test does not definitely rule out sensitization

3 Intravenous injections should be avoided as much as possible. Withdrawal of the syringe for evidence of blood may protect to some extent against accidental puncture of a vein but does not with certainty prevent this possibility

4 If no vein is punctured, the rapid appearance of a marked local reaction should invite caution. Epinephrine administered above the site of injection and application of a tourniquet may then aid in blocking absorption

5 Desensitization, according to Besredka, at short intervals is not a safe procedure for prevention of shock

6 Epinephrine, if given after symptoms have arisen, does not necessarily protect against the fatal outcome of shock. In cases suspected of sensitization, it should be given admixed with the antigen

ANEMIA, PERNICIOUS, Macrocytosis and Erythrocytes and Achlorhydria In, Haden, R. L. J. A. M. A. 98 202 1932

An increase in size of the average erythrocyte, best indicated in terms of volume, is the most constant and characteristic finding in the blood in the presence of pernicious anemia. An increased volume index was found in every patient in this series.

Free hydrochloric acid is seldom if ever found in the gastric contents of a patient with idiopathic pernicious anemia. An achlorhydria was demonstrated in every one of the 152 patients in this series.

The mean corpuscular volume may be quite large even with a relatively high count, therefore it does not vary with the red cell count.

If the deficiency which is responsible for the disease is adequately supplied, the cells return to normal size. The first indication of a relapse or a lack of a sufficient quantity of the missing principle is an increase in the volume of the red cells.

Macrocytosis may occur in the presence of conditions other than pernicious anemia but was found only 9 times in a study of 411 patients and normal individuals.

Achlorhydria is a frequent finding in various clinical conditions especially in the age period in which pernicious anemia is most common.

A combination of macrocytosis of the erythrocytes and achlorhydria is seldom if ever found, except in the presence of pernicious anemia.

The finding of an absence of free hydrochloric acid on gastric analysis and an increased mean corpuscular volume or plus volume index is a practically constant finding and one that is necessary for the diagnosis of active pernicious anemia as demonstrated it is almost pathognomonic of the disease.

AGRANULOCYTOSIS, Experimental, Fried, B. M., and Damashek, W. Arch. Int. Med. 40 94 1932

The purpose of the present study has been to determine the possible similarity between the blood picture as seen in agranulocytosis in man and that found in a form of experimental sepsis in rabbits.

The results have shown that there are close similarities between the agranulocytosis resulting from the hematogenous infection of rabbits with *Salmonella supestrifer* and that observed in cases of agranulocytic angina in man. Thus, the reaction in severe cases of human agranulocytosis corresponds to that of the animals that received overwhelming doses of bacteria, i. e., a persistent neutropenia and an intense necrosis of the bone marrow without signs of regeneration. A close similarity likewise exists between the "recovery phase" seen in the circulating blood in clinical agranulocytosis and that disclosed in the circulation of rabbits that were infected with relatively small doses of bacteria, i. e., a marked histiomonocytosis.

Incidentally, Schilling's clinical concept of "regenerative" and "degenerative" types of polymorphonuclear "shift" was confirmed by these experiments.

LANTERN SLIDES, A Simple, Quick, and Inexpensive Method Preparing, Reid, P. E. *Science*, 74: 418, 1931

Take a plain glass lantern slide, thoroughly clean it and allow to dry. When the slide has become quite dry apply a thin coat of albumen from a fresh egg and again allow the slide to dry. A smooth brush is essential in getting the coat of albumen evenly placed to avoid a streaked appearance when done.

As soon as the albumen has completely dried, place the plate with its coated side uppermost, over the diagram or other copy, and trace on the slide with India ink. The width of the lines may be varied by using pens of different sizes. Colored effects may be added in the same manner except that inks made from aniline dyes (such as the common writing fluids) should be used. The pigmentation in colored India inks make them all appear black on the screen.

Mount in the usual manner after the ink has dried by placing the newly made plate face down on another clean slide and fastening together with the usual lantern slide material or with adhesive tape.

These slides are not temporary as might be supposed but may be left in the lantern for long periods of time without injury in spite of the intense heat of some lanterns.

BACTERIA, Pure, Smooth and Rough Colonies at Will, Quirk, A. J. *Science* 74: 461, 1931

Make a needle transfer of the culture (broth, agar slant, or dispersed material) to a P_H 60 and a P_H 70 beef infusion broth tube. Then make a second dilution tube from each P_H grade of the (1) seeded broth tube. Hold the (2) dilution broth tubes in both P_H grades for 18 or 24 hours at room temperature.

After this growth period again make (1) and (2) dilutions from each P_H grade of the young culture to a corresponding P_H grade of broth. From the last (2) dilution broth tube transfer to a melted P_H 60 and P_H 70 beef infusion agar tube for poured plate examination.

This modified technic produces on the plates pure culture of the S or R colonies.

This technic involves three factors necessary to assure the pure S and R colonies. They are as follows:

- (a) Dilution before and after the young growth period of the organism
- (b) Young culture
- (c) P_H of the culture medium

For the smooth colony use only P_H 70 medium. For the rough colony use only P_H 60 medium.

The S colony is virulent, the R colony is avirulent.

Attention is called to the fact that an interchange of P_H grade from broth to agar plate may result in intermediate types with a corresponding interference in demonstration of virulence and nonvirulence on the host plant.

TUBERCULOSIS, Direct Culture of B. Tuberculosis from the Blood, Kren, O., and Lowenstein, E. *Klin. Wchnschr.* 10: 974, 1931

Preparation of Culture Medium. Five unbroken eggs are placed in 5 per cent solution of washing soda for 30 minutes, then rinsed in running water for 20 minutes then soaked

in 1 1000 bichloride solution for 20 minutes and again rinsed in water, and finally with 70 per cent alcohol

The following solution is prepared

Mono potassium sulfate	10 gram
Sodium citrate	10 gram
Magnesium sulfate	10 gram
Aspiragin	30 gram
Glycerine	600 cc
Distilled water	10000 cc

To each 150 to 160 cc of this solution add 6 grams of potato meal and 12 cc of glycerine. This mixture is boiled for 15 minutes and then placed in a 56° C water bath for 1 hour with frequent shaking.

The entire contents of four of the prepared eggs are now added and also the yolk only of the fifth egg. The mixture is then shaken vigorously with glass beads and 5 cc of 2 per cent sterile aqueous solution of Congo red. The medium is then filtered through sterile gauze and distributed in sterile tubes.

The tubes are placed in a thermostat in a slanting position and the medium coagulated after which it is sterilized in the Arnold for 1 hour on two successive days. The tubes are finally incubated 48 hours at 37° C as a check of their sterility.

Collection and Inoculation of Blood. From 8 to 10 cc of blood are drawn by venipuncture with a syringe into which 2 cc of sterile 5 per cent aqueous solution of sodium citrate have previously been drawn.

The blood is expelled into a sterile tube, centrifuged, and the plasma drawn off and discarded by means of a sterile pipette.

To the sedimented corpuscles add 5 cc of sterile 5 per cent acetic acid and stir with sterile precautions and again centrifuge. Withdraw the supernatant fluid with a sterile pipette and wash the mass twice with sterile distilled water.

The final sediment is now distributed over the surface of 4 to 6 slants being well spread and the tubes allowed to stand until the film of inoculum has dried. The tubes are then sealed with sealing wax and incubated at 37° C. Strictly aseptic technique must be observed throughout the whole procedure.

If the blood is received in a nonsterile condition it should first be shaken with 15 per cent sulphuric acid, allowed to stand 5 minutes, washed twice with sterile water, and the sediment thus obtained used for inoculation.

PNEUMOCOCCI, Immediate Typing of, Armstrong, R. E. Brit M J 3703 187, Jan 30, 1932

A method is described whereby pneumococcus typing may be carried out directly from the sputum.

A Gram stained smear is first examined to determine that pneumococci are present and also to gain an idea of the general character of the bacterial flora.

Selected flakes of sputum are then placed on each of four slides marked I, II, III and C (control).

A large drop of the respective type serum is placed next to the sputum, a drop of normal saline being used for the control. The sputum is then mixed with the serum, a coverslip applied and the preparations are allowed to stand while the Gram preparation is being examined.

Positive reactions are very striking and consist of a marked increase in the size of the pneumococci which also take a ground glass appearance and appear surrounded by a highly refractive peripheral zone.

The reaction may sometimes be delayed for as long as 15 to 20 minutes but is highly accurate when checked by the more elaborate methods.

The accuracy of the method is corroborated and highly commended by Logan and Smeall (Direct Method of Typing Pneumococci Logan W P and Smeall J J Brit M J 3703 188 Jan 30 1932).

Because pneumococci may be unevenly distributed in the sputum these latter authors, however, prefer to conduct the test with a saline emulsion of the sputum

MUSEUM TAGS, Chemical Proof, Schmidt, K P Science, 73 1931, 23, 1932

The paper known as Dennison's fiber proof paper was devised especially as a chemical proof paper for laundry tags. It appears to be a paper impregnated with albumin, which is subsequently hardened by treatment with formaldehyde. This paper comes in 20" x 24" sheets, somewhat variable in thickness. It does not soften in water, alcohol or formalin solution.

The 20" x 24" sheets in practice, are cut into $\frac{7}{8}$ " strips. These are printed with rules set $\frac{1}{4}$ " apart. Numbers are then stamped into the paper, to a depth of about half the thickness of the stock, by means of an automatic numbering machine. The printed rules serve as guides, so that the finished tag measures $\frac{1}{4}$ " x $\frac{7}{8}$ ". These impressed numbers are then inked by hand with Higgins waterproof drawing ink, to increase the legibility of the numbers, and dried. The numbering machine perforates the strip opposite each number, and the number strips next have the strings attached. The individual tags are then cut from this strip as wanted.

TUBERCLE BACILLI, Stain for Non Acid Fast Bacilli and Granules, Alexander, E G Science 75 1937, 197, 1932

Smears are stained with carbol fuchsin and decolorized as usual after which one of the counterstains described below is applied.

COUNTERSTAIN METHOD I

Flood the smear with 8 drops of Loeffler's methylene blue. Add immediately 6-8 drops of 0.05 per cent NaOH from a medicine dropper. Move the slide gently from side to side to mix, and let stand two to three minutes. Wash with tap water, dry and examine under the oil immersion lens. The contrast between the red acid fast tubercle bacilli and the blue non acid fast tubercle bacilli is striking.

COUNTERSTAIN METHOD II

Flood the smear with 8 drops of 1 per cent aqueous crystal violet solution. Add immediately 6-8 drops of 5 per cent NaHCO_3 . Move the slide gently from side to side to mix, and let stand not more than 2 minutes. Wash in tap water, apply Gram's iodine for 2 minutes, wash, and decolorize 20-30 seconds with a mixture of equal parts of acetone and 95 per cent alcohol, wash dry, and examine under the oil immersion lens. The non acid fast bacilli appear violet. In addition the "Granules" in the red acid fast bacilli and in the violet non acid fast bacilli stand out prominently as violet black bodies.

MONONUCLEOSIS, INFECTIOUS, Presence of Heterophile Antibodies In, Paul, J R, and Bunnell, W W J M Sc 183 90, 1932

The following technic was used.

The authors have employed the methods used by Davidsohn for determining the presence and titer of sheep cell agglutinins and hemolysins. The technic is quite simple.

Sheep Cell Agglutinins Sera, obtained as for a Wassermann test, were inactivated for 15 minutes at 55° C. Dilutions of inactivated sera, ranging from 1 to 4 to 1 to 32 (or higher if the heterophile antibody content was suspected to be present in unusual concentration) were set up in 0.5 cc portions. To these 0.5 cc of a 2 per cent suspension of sheep cells were added followed by 1 cc of salt solution, thus bringing the total volume in each tube to 2 cc. The test tubes were shaken and placed in the water bath at 38° C for 1 hour, left in the icebox overnight and on the following morning were read, after each tube had been inverted three times with its mouth covered by the finger tip.

For the sake of conformity with previous work, the readings have been recorded in terms of the original dilution of the 0.5 cc of sera added to each tube. With the subsequent addition of 0.5 cc of the suspension of sheep cells and 1 cc of saline, the dilution of serum becomes much higher so that actually the tube designated 1 to 4

contains 0.12 cc of serum in 2 cc of cell suspension that designated 1 to 8 contains 0.06 cc of serum in 2 cc of suspension. Legends for recording the readings are given in the following terms: — Firm disk, ++ disk easily broken into large flakes, - fine agglutination, ± barely perceptible, but definite agglutination.

Sheep Cell Hemolysins. In the observations recorded below all of the results have been given in terms of sheep cell agglutinations. Sheep cell hemolysins were found to parallel the agglutinin content with such regularity that the former have not been recorded. In many of the earlier determinations both tests were run and were found to serve as a rough check, one for the other.

Hemolysin tests were run as follows. Original dilutions of 0.5 cc of inactivated sera, similar to those used in the agglutinin tests, were employed. To these 1 cc of guinea pig complement in a dilution of 1 to 30 was added then 0.5 cc of a 2 per cent suspension of sheep cells, followed by 1 cc of saline thus bringing the total volume to 3 cc in each tube. The tubes were shaken and placed for 1 hour in a water bath at 38° C and read.

They conclude from their studies that

1 Heterophile antibodies, demonstrable in the form of sheep cell agglutinins, have been recorded in rather high concentrations in the active stages of 4 cases of infectious mononucleosis.

2 Apart from cases of serum disease, and one notable exception the authors have failed to note this finding in a large series of cases representing a variety of clinical conditions, including cases of Vincent's angina, lymphatic leucemia and other blood dyscrasias.

3 There would seem to be two possible explanations for this finding: (1) that the unknown agent responsible for infectious mononucleosis contains the heterophile antigen, (2) that we are dealing with an example of isoagglutinin production elicited by abnormal cells, which are present either in the blood or elsewhere during active stages of the disease.

BRUCELLA INFECTIONS, The Endermic Reaction In, Leavell, H. R., and Amoss, H. L. Arch Int Med 48 1192, 1932

The following conclusions are advanced:

1 The endermic reaction is of value in the diagnosis of undulant fever, particularly in cases in which no agglutinins for *Brucella* are present in the blood serum and in which *Brucella* cannot be grown on culture of the blood, urine, stools or bile.

2 The intracutaneous test is not definitely specific. Although the result is generally positive in undulant fever, it is not frequently positive or highly suggestive, in controls.

3 Representative strains of several different types of *Brucella* should be used in making the test. The interpretation of the test should be based on all the reactions rather than on a single one.

4 Extracts of *Brucella* prepared according to the methods of Lancefield and of Ando for securing the soluble specific substance gave no more specific results in our cases than did the simple saline suspensions and extracts.

5 It is probable that an extract of *Brucella* prepared by prolonged shaking of a saline suspension followed by centrifugation to remove most of the organisms gives fewer false reactions than other preparations. But such a preparation does not always give a positive reaction in undulant fever.

6 Heat killed bacterial suspensions seem to have more specific action than bacterial filtrates.

7 It is of value to titrate the degree of the endermic reaction by using varying dilutions of suspensions of the strains being tested as in the tuberculin reaction.

ANEMIA, PERNICIOUS, Pigment Metabolism and Destruction of Blood In, Farquharson, R. F., Borsook, H., and Goulding, A. M. Arch Int Med 48 1156 1932

The authors thus summarize their studies:

1 In patients with Addison's "pernicious" anemia in a state of relapse the excre

tion of urobilinogen is greatly increased and may be several times the normal value. In severe cases the amount excreted daily is equivalent to 8 to 13 gm of hemoglobin, amounting in some instances to more than one tenth of the total blood hemoglobin of the body.

2 Following liver therapy and beginning about the peak of the reticulocyte crisis, there is always a sharp decrease in the excretion of urobilinogen, which falls in a few days to a level within normal limits.

3 In cases of pernicious anemia showing little anemia, with general macrocytosis but little microcytosis or poikilocytosis, the excretion of urobilinogen is increased somewhat above a high normal value. With liver therapy the excretion of urobilinogen falls after a low reticulocyte response and is always normal when the blood picture has become normal.

4 The amount of plasma bilirubin in pernicious anemia varies in the same direction as the excretion of urobilinogen and opposite to that of the red blood cell count. It is highest in those patients who are critically ill. When it is high it falls to a normal level during the reticulocyte response.

5 In severe cases of pernicious anemia, urobilin is frequently found in the urine. Large amounts are present only when patients are very ill. Its excess then is attributable, in part at least, to altered liver function.

6 The disturbance in blood pigment metabolism in pernicious anemia is probably due to an abnormality of the red blood cells in virtue of which they suffer early destruction. The abnormal red blood corpuscles present in relapse are the ones most affected. Adequate liver treatment, promoting the production of normal red blood cells, retards the rate at which destruction of the blood occurs and allows the blood picture to become normal.

BLOOD SUGAR. Following the Rectal Administration of Dextrose, Scott, E. L., and Zweighaft, J. F. B. Arch. Int. Med. 49: 221, 1932.

It has not been possible to demonstrate a rise in the blood sugar curve as a result of administering dextrose in retention enemas.

The slight drop that the curves show may be due to a stimulation of pancreatic activity brought about by the absorption of a slight amount of dextrose, or, more probably, to chance variation.

A variable and frequently considerable amount of dextrose administered by enema may be recovered from the stools after two and one-half hours.

TUMORS, a Coaguloflocculation Test for Malignant, Weiss, E. Arch. Path. 13: 106, 1932.

The details of the method follow.

Glassware and Apparatus—For the measuring of serum and antigen, 0.2 cc pipettes are required. For the dilution of the serum, 1 cc serologic pipettes are most satisfactory. Wassermann tubes with a diameter of $\frac{1}{2}$ inch (1.27 cm) and 4 inches (10.16 cm) long appear to be very suitable. Similarly, tubes of the same diameter, but 2 inches (5 cm) longer, are useful in instances in which deeper submersion in the water bath is necessary or more desirable. The glassware should be rinsed with warm water and placed overnight in a cleaning solution (equal parts of a 6 per cent solution of sulphuric acid and a 6 per cent solution of sodium or potassium dichromate) then rinsed several times with distilled water and allowed to dry. Racks of Wassermann tubes with two or three rows of holes are most useful. A water bath easily adjustable to from 54° to 55° C and maintaining a uniform temperature is required. An interval timer with an automatic alarm is convenient on account of the brief incubation.

Blood Serum—Serums should be fresh and thoroughly centrifugated, they do not require inactivation. Serums that are rich in lipoids, contaminated or hemolytic should be eliminated as unsatisfactory for the test.

Serum Diluent—Distilled water is used instead of salt solution.

Antigen—Plain alcoholic beef heart antigen. Beef heart is freed from all fat and finely ground. One hundred grams are extracted with 1,000 cc of 95 per cent alcohol for

three days at 37° C and then left overnight at room temperature. The extract is then filtered and kept in dark bottles, closed with rubber corks. The antigen is then ready for use.

Sodium Chloride Solution—Sodium chloride solution is prepared by dissolving 40 gm or chemically pure sodium chloride in 100 cc of distilled water. A saturated solution of sodium chloride is equally satisfactory if filtered before it is used.

PROCEDURE

Method of Calculating Dilutions of Serums Previous to Use in Test—The dilution of serums is carried out according to the percentages of hemoglobin. Tallquist's scale is most commonly used in the determination of hemoglobin, it is disadvantageous because it is less accurate than other procedures. In order to have uniformity in the results obtained, Dare's hemoglobinometer is used as a standard. To the percentage of hemoglobin obtained with Dare, 10 is added and the sum is divided by 20, which gives the dilution for the respective serum. (For instance, if the reading is 70 per cent, 10 is added, giving 80. This divided by 20 equals 4, the dilution of the serum in this case would be 1:4.) Tallquist's scales can be used if their average reading is previously compared with Dare and the difference taken into consideration in the calculation of the dilutions of the serums. (For instance, if the Tallquist scale shows a 10 per cent higher reading than the Dare nothing is added to the hemoglobin reading before dividing with 20. If the Tallquist reading is 15 per cent or 20 per cent higher than Dare, 5 or 10, respectively, is subtracted from the reading before dividing with 20.) When the Dare reading is 40 per cent or less, the serums should be diluted only to 1:2.5.

Titration of Antigens Previous to Use in the Test—The selection of the proper amount of antigen is based on its reaction with malignant and syphilitic serums. The syphilitic serums are more sensitive than the normal serums and are therefore more valuable for the titration. Properties similar to those of syphilitic serum are observed also in the serum in jaundice. All antigens are titrated in the following manner. In each of two rows of a rack, eight tubes are placed. Increasing amounts of undiluted antigen (0.12, 0.14, 0.16, 0.18, 0.20, 0.22, 0.24, 0.26 cc, etc.) are placed in the corresponding tubes of both rows. In each tube of the first row 0.6 cc of the diluted (according to the procedure described) malignant serum is added, and each tube of the back two receives 0.6 cc of the similarly diluted syphilitic serum. The tubes are thoroughly shaken and then placed in a water bath for five minutes at from 54° to 55° C. After the incubation, the content of each tube is slowly diluted with 2.5 cc of saturated sodium chloride solution, and the results are recorded. Usually, the smaller amounts of antigen cause turbidity in the syphilitic and malignant tubes, "lower non-specific zone." Larger amounts cause turbidity in the syphilitic tubes while in the malignant tubes the more or less coagulated serum floats on the surface of the saline solution which contains floccules. This is the "specific zone." Still larger amounts of antigen cause a positive reaction (flocculation) both in the malignant and in the syphilitic tubes "upper non-specific zone." The largest amount of antigen that causes only turbidity in the syphilitic tube and a distinct coaguloflocculation in the malignant tube is selected as the proper amount for the test (= titer). The titrated amount of antigen should also be tested with icteric and anemic serums. The titer remains the same for an indefinite period if the antigen is properly preserved.

Outline Test—Wassermann tubes are placed in two rows in the racks. The tubes of the first row are used for the main test with the unknown serums and also for the malignant syphilitic icteric and anemic controls. The last tube in the first row contains the antigen control. The tubes in the second row serve as the serum controls for the unknown serums and also for the malignant syphilitic icteric and anemic serums. The titrated amount of the undiluted antigen is placed in each tube of the first row. The corresponding amount of distilled water is placed in all tubes of the second row. Six tenths of a cubic centimeter of each diluted serum is added to one tube in the first row and an equal amount of the same serum to the tube behind in the second row. Six tenths of a cubic centimeter of distilled water (instead of serum) is added to the antigenic

control The ingredients of malignant, syphilitic and icteric controls and their serum controls should be the same as those used for the unknown serums All tubes are then shaken and placed in a water bath at from 54° to 55° C for five minutes After the incubation, 2.5 cc of a saturated, or 12.5 per cent sodium chloride solution is slowly added to each tube, and the results are read

If the required amount of the unknown serum is not available, the test may still be performed successfully if the remaining constituents for the reaction are decreased proportionately

Controls—The following controls are necessary each time the test is carried out (1) antigen control, (2) serum control (each serum should have a serum control), (3) malignant, syphilitic, icteric and anemic controls

Interpretation of the Results—The controls should be examined before making readings of the unknown serums The malignant control should show a thick layer of coagulated serum floating on the surface of the salt solution, which contains many large floccula All other controls should remain uniformly turbid One tube is read for each unknown serum Tubes showing the same reaction as the malignant control are read as strongly positive Tubes with a distinct flocculation without showing in addition a layer of suspended coagulated serum on the surface of the saline solution are read as weakly positive Uniformly turbid tubes are read as negative Tubes with a doubtful flocculation are also read as negative and the test should be repeated

Sources of Error—(1) The used serum is hemolytic, contaminated or inactivated, or contains an excessive amount of lipoids, (2) the serum is not properly diluted, (3) some other diluent was used instead of water, (4) the antigen was not properly prepared or preserved, (5) the antigen was not accurately titrated, thus causing either nonspecific reactions or a low percentage of specific reactions, (6) the antigen was not vigorously mixed with the diluted serum, (7) fallacies occurred in the reading of the temperature and in the duration of incubation, (8) another reagent than the saturated salt solution was used for the dilution of the serum antigen mixtures after the incubation, (9) the tubes were shaken after addition of salt solution, thus rendering the reading difficult

REVIEWS

Books and Monographs for Review should be sent direct to the Editor,
Dr Warren T. Vaughan, Professional Building, Richmond, Va

Ergebnisse der medizinischen Strahlenforschung

TO THOSE familiar with German 'ergebnisse' nothing more need be said. These statelv annual volumes of about 700 pages (7 by 10½ inches) are of the usual high standard. They are especially well illustrated as one might expect from the nature of the subject. Volume III has monographs on the x-ray diagnosis of pulmonary echinococcosis and of conditions in the nose and accessory sinuses, the ear, petrous bone, bronchi, esophagus, intestine, appendix, gall bladder, also radiation and the normal skin, light and metabolism, radiation of adenoids and diathermy in gynecology. Volume IV contains ultraviolet rays and resistance to light, pigment and light resistance erythema from light, principles of the biological treatment of carcinoma and diathermy in ear, nose and throat work. Volume V, the cascade stomach, the physics of light therapy, mutation by radiation, radium surgery, bone tumors and work with monochromatic light.

Ueber die Akute und Chronische Gelbe Leberatrophie mit Besonderer Berucksichtigung ihres Epidemischen Auftretens in Schweden im Jahre 1927

THIS monograph reports 163 cases of yellow atrophy of the liver, 97 of which occurred in epidemic form in Sweden in 1927. There is a good epidemiological account of the epidemic and a well illustrated chapter on the pathologic anatomy of the disease. Because the disease so closely resembles icterus catarrhalis and because it may occur in epidemic form, the author feels that it must be due to some specific causative agent not yet discovered.

Allergie des Lebensalters—die bosartigen Geschwulste

SOME years ago von Pirquet observed that in his pediatric clinic in Vienna there were many more girls than boys under fourteen years of age who showed tuberculous infection. Wondering if this were true generally, he turned to a report of the English vital statistics for 1910 which by chance had been given to his clinic and made a graph of the deaths from tuberculosis by age and sex. He found from these figures also that the curve for boys rises several years later than it does for girls. In this way he became interested in vital statistics and made an extensive study of the excellent series for England and Wales. He hoped to cover the whole subject but the part on malignant tumors was the only part complete at the time of his death and this, put through the press by his friend, Dr Herbert Orel, forms the present volume.

Part one consists of large numbers of curves showing deaths from tumors according to age, sex, and site of tumor. He finds that these graphs are of different types, that is, for example, the age incidence of tumors differs for different parts of the body or for different organs and by bringing the graphs of similar types together into groups he shows the reactivity or susceptibility of the parts of the body for tumors according to age or as he calls it the

*Ergebnisse der medizinischen Strahlenforschung. (Results of the investigations of medical radiation including x-ray diagnosis and the therapeutic use of roentgen rays, radium and light.) Vol III 1925 IV 1930 V 1931. Georg Thieme Leipzig.

**Leber die Akute und Chronische Gelbe Leberatrophie mit Besonderer Berücksichtigung ihres Epidemischen Auftretens in Schweden im Jahre 1927. (Acute and Chronic Yellow Atrophy of the Liver With Especial Reference to Its Appearance in Epidemic Form in Sweden in 1927.) By Prof. Dr. Hilding Bergstrand. Published by Georg Thieme Leipzig 1930.

*Allergie des Lebensalters—die bösartigen Geschwülste. The Allergy of Age—the Malignant Tumors. By Dr. Clemens Pirquet. Published by Georg Thieme Leipzig 1930.

allergy of age. In this way he makes what he considers natural groups of tumors as follows: those of the abdomen, thorax, mouth, male sex organs, female sex organs, skin, and other organs.

Human Biology and Racial Welfare

THIS is yet another volume in which Edmund Cowdry demonstrates his abilities as an editor and impresario. First, he has good ideas concerning subjects that would be of special interest, and second, he has the knack of persuading men who are peers in their respective fields to collaborate with him. As the title would indicate, this is a volume of no mean ambition. Indeed, it is a compilation of the frontiers of thought and investigation in the social sciences and in the study of man and his environment.

The start is breath-taking enough, dealing as it does with what we know of the whole universe, particularly of the possibilities of life in the universe. Following this are several chapters on the origin of man and evolution—individual, mental, and social. Then, comes a discussion of man himself as a physiological unit, built up from the cells or vital units, through cell aggregates, and the importance of the vascular system, later of the nervous system, and on through the more highly complex discussion of sex integration.

Part Four deals with the effects upon man of his environment and Part Five ventures some prophecies as to the future. The list of contributors is breath-taking and the scope of the discussion is almost truly universal as it relates to man.

This is a monumental work, the kind that a really intellectual man likes to have near him for piecemeal reading, with the assurance that all that he can absorb therefrom is authoritative and will keep him conversant with the most advanced thought of the day in the study of mankind.

You have seen the advertisement of the bashful young man who never had anything to say at social gatherings, but who after taking the Benjamin Franklin home correspondence course in French astounded his friends by his subsequent ability to lead the field in any intellectual conversation. The reviewer ventures to say that anyone who can readily assimilate all that is in this volume would be able to requit himself regally thereafter in any really intellectual drawing room levee.

Not all of the contributors have been universally successful in acquitting themselves equally well in grading down the tempo of their discussion to the speed of the average reader, not specialists in their own subjects, and as a consequence parts are rather hard reading, but the majority have achieved this admirably.

American Physicians and Surgeons†

THIS book is a really laudable effort to establish a reliable Who's Who in American Medicine. Directories of American physicians and surgeons have appeared in the past but a cursory examination almost always reveals that the price of having one's name appear therein is the purchasing of a copy of the directory.

This volume, issued by the publishers of "The American Bar" which has been a recognized Who's Who in the legal profession for several years, has been developed strictly on a who's who basis. The selection of names has been based on membership in special societies and direct reference to the prominent physicians of each community.

As is inevitable in a first edition, the names of a number of outstanding men in different communities have been left out. But it is better to have errors of omission than of commission. In those cities with which the reviewer is well acquainted there have been practically

*Human Biology and Racial Welfare. 28 Authors. Edited by Edmund V. Cowdry, Professor of Cytology, Washington University, St. Louis. With an introduction by Edwin F. Embree. Illustrated. Cloth. Pages 612. Paul B. Hoeber, Inc., New York, 1930.
†American Physicians and Surgeons. A Biographical Directory of Practicing Members of the Medical Profession in the United States and Canada. Including supplements in which are listed and classified the leading hospitals, sanitariums and health resorts of both countries. Prepared by James Clark Fifield, Editor of The American Bar. Leather. Pages 1737. The Midwest Company, Minneapolis, 1931.

no names included who did not deserve the recognition. The others who should be in can easily be added in the next edition.

Of course there are many who did not fill out the biographical data requested before the publication of the volume. Their names however appear with as much information as the editors could cull from the directory of the American Medical Association and the list of the special societies.

The price of the volume is high but we presume that it must be so in a first venture of this sort in which the outcome is uncertain.

We hope that the editors will succeed, will adhere to their high standard which they have set for themselves, and will be able before long to reduce the price of the volume.

Symptoms of Visceral Disease

OCCASIONALLY a medical prophet, far ahead of his times, will conceive and develop a new interpretation of disease or of certain diseases. He may tell his story repeatedly to an unheeding multitude, unwilling or unequipped to grasp the significance of the story. If he is so unfortunate as to pass on before the average doctor has gained an adequate comprehension of his theory, all of his labors will be lost for a time until some new thinker, possibly many years later, will rediscover the idea. Such a man was Sir James MacKenzie and such an idea was his principle of the reflex arc.

Why is it that the average doctor shuns all invitation to any profound study of organic neurologic reflexes and responses? Is it because as a rule he is poorly trained in neurology and prefers to leave the subject for a specialist? Scarcely any field of medicine is more nearly a true science than that of organic neurology and neurologic localization, and diagnosis is a very simple thing provided one really knows the anatomy of the central nervous system. All that is required is a little real study.

The trained neurologist is too often so occupied with his studies of organic disease of the central nervous system and peripheral nerves, that he has scant time to give to the neurologic factors in functional visceral reflexes.

It is the internist who should be primarily interested in the latter, for as a rule these reflexes are manifested not so much as disease of the nervous system, but more as disease in the internal organs which only occasionally produces functional neurologic manifestations.

While MacKenzie was unfortunate in that he did not live to see a general acceptance of his principle of the reflex arc, he was fortunate in having a disciple able to comprehend his teachings and to carry on. The volume by Dr. Pottenger under review is however, not a mere continuation of MacKenzie's teachings. It is a summary of a life study by the author into which he has incorporated much of MacKenzie's teachings and in which he has correlated these with his own entirely original, and different in many respects, concepts of the functional neurologic reaction in visceral disease.

Years ago Dr. Pottenger became enthused over a study of the autonomic nervous system with its two antagonistic subsystems, and from his investigations he rapidly popularized the concept of the function of the vegetative nervous system. While his first interest was in its action as related to pulmonary disease he has extended this to visceral disease in general.

The volume does not lend itself well to abstraction, but we may state without hesitation that it is the most comprehensive and intelligible of the available treatises on the function of the vegetative nervous system in its relation to the remainder of the nervous system and to organic visceral disease as applied to clinical medicine. The fact that it has already reached its fourth edition would indicate that there are many doctors who do not possess the illusion that neurologic studies are too dry or too deep.

*Symptoms of Visceral Disease. A Study of the Vegetative Nervous System in its Relationship to Clinical Medicine. By Francis Marion Pottenger, A.M., M.D., LL.D., F.A.C.P., Medical Director, Pottenger Sanatorium for Diseases of the Lungs and Throat, Monrovia, California. Fourth Edition. With 57 text illustrations and 10 color plates. Cloth. Pages 426. The C. V. Mosby Company, St. Louis, 1930.

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EDITORIAL

Malignancy in Radioactive Persons

Radium poisoning as a new and exceedingly dangerous occupational disease was first brought to light by the studies of Maitland and his associates,¹ in 1925 as a result of investigations conducted upon individuals engaged in the manufacture of luminous watch dials

From these studies it was shown that radioactive substances entering the body by ingestion, absorption through the skin, or inhalation were eventually stored as an insoluble sulphate, in particulate or colloidal form, in the main organs of the reticuloendothelial system and especially in the long bones from which latter site in particular they continued to emit their characteristic emanations

It has been demonstrated also that as little as one one-hundred-thousandth of a gram of radioactive substance so deposited continues to emit alpha emanations at an incredible speed and in innumerable numbers for indefinite periods of time so that it has been estimated, for example, that assuming such a deposit to have occurred in the body in 1925, the skeleton will still be emit-

ting 185 000 alpha particles per second at a speed of 18,000 miles per second in the year 3491 A D

As the alpha particles are probably the most potent and obstructive agents known to science, the importance of this industrial hazard becomes at once apparent

As a result of this constant and continual radiation from the deposits in the bones, the bone marrow, and the blood-forming organs, periods of irritative stimulation and overstimulation are followed by periods of exhaustion, the symptomatic expressions of which are seen in necroses of the jaw and in the development of a leucopenic anemia of regenerative type, resistant to all means of treatment and ultimately as well as often rapidly, fatal

The bone necroses resulted from the occurrence of an intense osteitis and when, as in the jaw, a superimposed bacterial invasion occurred necrosis was a natural aftermath

These were the early symptoms and it has remained for continued study and observation the results of which are now reported by Martland² to establish the fact that a late result of the deposit of radioactive substances in the human body is the inevitable development of rapidly growing embryonal or anaplastic osteogenic sarcomas

Six deaths are now reported from this cause and several other cases are on record in which death has not as yet occurred but in which a fatal outcome is inevitable

The sequence of events in the production of these terrible sequelae is believed by Martland to be, first, a hyperplastic marrow irritation, succeeded by a period of replacement fibrosis which in its initiation is very cellular and as a product of which osteogenic sarcoma occurs

For the first time in the history of medicine the story of the effect of the internal bombardment of human tissues by the alpha particle has been recorded, an imposing list of lesions irritative hyperplasia and compensatory stimulation of the bone marrow of a very primitive type, leucopenias, mild anemias of the pseudoaplastic type, fatal anemias of the regenerative or megaloblastic type with leucopenias approaching an agranulocytosis, but with no evidence of hemolysis and rarely marked hemorrhagic tendencies, replacement fibrosis with production of a radiation osteitis, necrosis of the jaw due to a superimposed infection upon a radiation osteitis, crippling and deforming bone lesions due to healing radiation osteitis, with coxa vara, deformities of the spine, spontaneous fractures, etc., a packing of the bone marrow with primitive stem cells resembling leucemoid state, the possibility of the development of myeloid leucemias and even multiple myelomas, and finally osteogenic sarcomas

Martland also very pertinently emphasizes the relation of his findings to the many radioactive waters emanators activators and so on which are offered for sale to a credulous public

Even though their content of radioactive substances may be so slight as to render any good effects from their use purely psychic the studies above summarized clearly show that extensive lesions of serious character follow

the implantation in the body of incredibly small amounts of radioactive substances. Even less than one-half a microgram may be dangerous in Martland's opinion.

That the conception of radioactive waters as a dangerous source of deposit of such substances in the body is neither extreme nor fallacious is shown by the fact that persons ingesting them over a period of one or two years have been shown to have radioactive deposits in their bodies, demonstrated both by electrometric tests, and also by the occurrence of bone necrosis.

The study and the reports upon which it is based and from which it has developed, are of absorbing interest, not only as recording the story of a new and terrible industrial hazard, not merely as an outstanding scientific achievement, but also as suggesting new avenues for the experimental study of malignancy.

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—R. A. K.

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SYMPOSIUM ON HEMATOLOGY

THE TECHNIC OF A BLOOD EXAMINATION*

RUSSELL L HADEN MD CLEVELAND OHIO

IT MAY not appear to be necessary to preface a series of articles on hematology with one on the technic of a blood examination. Knowledge of the blood diseases is dependent however on the data obtained from a study of the blood. The presence of an anemia leucemia or other blood disease may be surmised but no clinician would hazard an absolute diagnosis or outline a course of treatment without the aid of the laboratory.

It is important to have at hand as complete laboratory data as possible and still more important to make sure that these findings are absolutely accurate. Too often the clinician is called upon to express an opinion based on blood films which are poorly made or unsatisfactorily stained or on a blood picture for which the data is incomplete or evidently inaccurate. The selection of the best technical methods is difficult for those who are not constantly studying the problems of hematology.

The above considerations are my excuse for attempting in this article to outline a scheme of blood examination and to suggest satisfactory technical methods for the elucidation of the simpler problems of hematology.

A routine blood count (red and white cell count hemoglobin estimation and differential count) is only a starting point for a more complete blood study and should be looked upon largely as a means of determining whether or not a complete blood study is indicated. In every case of anemia the following examinations should be done:

- 1 Red corpuscle count
- 2 Determination of the mass of packed corpuscles
- 3 Hemoglobin estimation

*From Cleveland Clinic

4 Calculation of indices

- a Volume index (erythrocyte volume relative to normal) or mean corpuscular volume
- b Color index (erythrocyte hemoglobin relative to normal) or mean corpuscular hemoglobin content
- c Saturation index (concentration of hemoglobin per unit volume of packed cells relative to normal) or mean corpuscular hemoglobin concentration

5 White corpuscle count

6 Study of stained blood film (size, shape, staining reactions and abnormalities of red cells, differential count of white cells, relative number of platelets)

7 Count of reticulocytes

8 Determination of bile pigment content of the plasma

These examinations are all necessary and are very easily done. I much prefer to make all examinations except the study of the stained film, on blood withdrawn from a vein. The blood film alone is made from a drop of blood obtained from the ear lobe or the finger tip. A simple method for the entire ex-

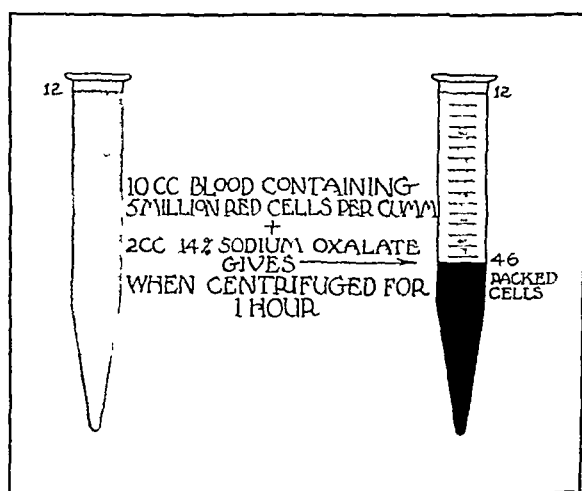


Fig. 1—Centrifuge tube used for determination of mass of red blood cells

amination is as follows. 20 cc of blood is withdrawn by means of a syringe, and exactly 10 cc is run into a 12 or 15 cc centrifuge tube, containing exactly 2 cc of 14 per cent sodium oxalate solution¹. This is mixed by inverting and is then spun in a large centrifuge for one hour at 2500 revolutions per minute. The remainder of the blood is added to an ounce bottle containing one drop of a 30 per cent solution of potassium oxalate. The latter specimen is used for the red and white cell count and for the hemoglobin determinations. The examinations are made as indicated below.

1 Red Cell Count—One source of inaccuracy in erythrocyte counts is the use of a hypotonic diluting fluid. I prefer to use a 0.9 per cent sodium chloride solution as the diluting agent. Accurate erythrocyte counts require much practice and experience on the part of the technician. It is absolutely necessary that accurately calibrated counting chambers and pipettes be used. These should be certified by the United States Bureau of Standards.

2 *Volume of Packed Red Cells*—This is read off directly from the tube after centrifuging. The volume is recorded as the number of cubic centimeters of cells per 100 c.c. of blood and in per cent of normal. The normal is calculated for each laboratory by determining by means of the centrifuge the number of cubic centimeters of packed cells per 100 c.c. of blood in normal individuals with a red cell count of 5 million cells per c.mm. With our present apparatus we have found 45 c.c. of cells per 100 c.c. of blood to be equal to 100 per cent (Fig. 1). For any given specimen of blood the number of cubic centimeters of packed cells obtained by centrifuging 10 c.c. of blood is read off on the

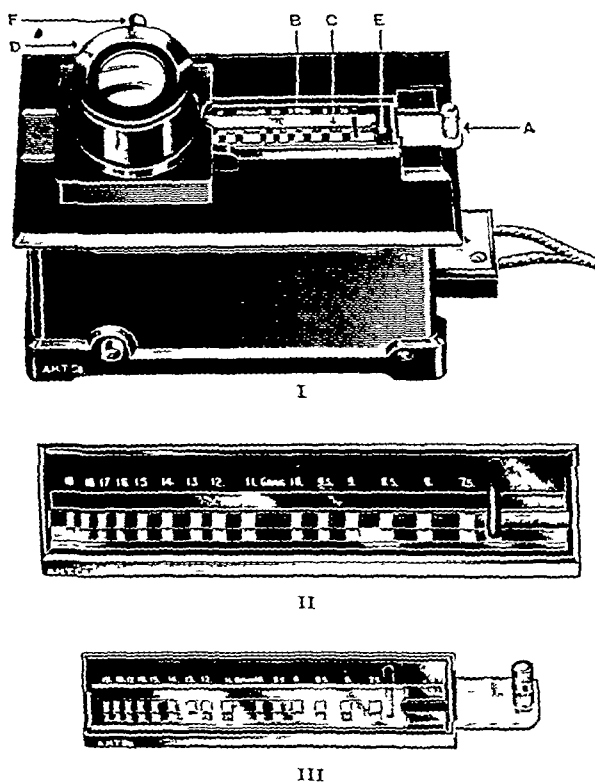


Fig. 2—Haden-Hausser hemoglobinometer. I Complete instrument. A movable carrier. B comparator slide. C cover glass. D reading microscope. E wedge-shaped channel. F light shutter. II Comparator slide. III Comparator slide with cover glass in metal holder.

tube and divided by 45 c.c. (or other figure determined as normal for the 10 c.c. of blood)

3 *Hemoglobin Estimation*—Accurate hemoglobin determinations may be made quite easily by the oxygen capacity method using the Van Slyke apparatus or by one of the non methods. Such procedures are not practical however in routine clinical work. The exact number of grams of hemoglobin present in any given blood is of no great clinical importance. It is exceedingly important however to determine the hemoglobin content relative to normal. This can be done simply if a hemoglobinometer reading directly in grams is used for

the determination. The new Sahli, the new Dacie, the Bausch and Lomb New-comer, the Klett, the old Miescher-vonFleischl, and the Haden-Hausser² instruments, all read in grams although no two give the same reading on the same specimen of blood. This makes little difference if every one in his own laboratory determines for the instrument used the average number of grams of hemoglobin per 100 cc of blood in normal individuals with a red cell count of 5 million per cmm, and takes this as 100 per cent. The results are then always reported, not in the absolute number of grams per 100 cc, but in per cent of normal. In normal individuals the color index is always 1.00 within the limits of error. By this method the percentage of hemoglobin for a given specimen of blood would always be the same in all laboratories although the actual number of grams of hemoglobin determined would be different in each. In our laboratories the Haden-Hausser hemoglobinometer (Fig. 2) which reads only in grams is used routinely and 15.4 grams of hemoglobin is taken as 100 per cent.

4 *Calculation of Indices*—By the methods outlined we are now able to determine accurately the red cell count and the packed cells in per cent of normal, packed cells (normal equals the number of cubic centimeters of packed cells found in 100 cc of normal blood with a red cell count of 5 million) and the hemoglobin in per cent of normal hemoglobin (normal equals the number of grams of hemoglobin found in 100 cc of normal blood with a red cell count of 5 million). Suppose for a given laboratory a specimen of normal blood with a red cell count of 5 million per cmm yields 46 cc of packed cells per 100 cc on centrifuging with an isotonic anticoagulant for one hour at 2500 revolutions per minute and contains 15 grams of hemoglobin per 100 cc, and a specimen of anemic blood with a red cell count of 1.5 million yields 18.4 cc of packed cells, and contains 6.0 grams of hemoglobin, then

(1) The volume index (volume of average cell relative to normal)

$$\begin{aligned} \text{of the normal blood} &= \frac{\text{Number of cc of packed cells found per 100 cc}}{\text{Normal number of cc of packed cells per 100 cc}} \\ &= \frac{\text{Number of red cells found}}{\text{Normal number of red cells}} \\ &= \frac{46}{46} = 1.00 \\ &= \frac{5,000,000}{5,000,000} \\ &= \frac{18.4}{46} \\ \text{of the anemic blood} &= \frac{18.4}{46} = 1.33 \\ &= \frac{1,500,000}{5,000,000} \end{aligned}$$

The mean corpuscular volume³ (the volume of the average red corpuscle in cubic microns) is calculated by dividing the volume of packed cells per 100 cc by the number of cells contained in 100 cc of blood. The result may be calculated in cubic microns by multiplying by 2 the volume of packed cells per 100 cc per 5,000,000 cells.

Thus the mean corpuscular volume of the normal blood = $46 \times 2 = 92$ cubic microns,
of the anemic blood = $\frac{184}{1,500,000} \times 2 = 61.3 \times 2 = 123$ cubic microns

$$\frac{184}{1,500,000} = 0.0001227$$

$$\frac{0.0001227 \times 2}{1} = 0.0002454$$

(b) The color index (amount of hemoglobin per cell relative to normal)

of the normal blood = $\frac{\text{Number of grams of hemoglobin found per 100 cc}}{\text{Normal number of grams of hemoglobin}} \times \frac{\text{Number of cells found per c mm}}{\text{Normal number of red cells per c mm}}$

$$\frac{15}{15} \times \frac{5,000,000}{5,000,000} = 1.00$$

of the anemic blood = $\frac{60}{150} \times \frac{1,500,000}{5,000,000} = 1.33$

$$\frac{60}{150} = 0.4$$

$$\frac{0.4 \times 1,500,000}{5,000,000} = 1.33$$

The mean corpuscular hemoglobin (the hemoglobin content of the average red corpuscle in micromicrograms) is calculated by dividing the hemoglobin in grams per 100 cc of blood by the number of cells contained in 100 cc of blood. It is simply calculated in micromicrograms by multiplying by 2 the number of grams of hemoglobin per 100 cc of blood per 5 million cells.

Thus the mean corpuscular hemoglobin of the normal blood = $15.0 \times 2 = 30$ micrograms
of the anemic blood = $\frac{60}{1,500,000} \times 2 = 20 \times 2 = 40$ micromicrograms

$$\frac{60}{1,500,000} = 0.00004$$

$$\frac{0.00004 \times 2}{1} = 0.00008$$

(c) The saturation index (amount of hemoglobin per unit volume of cell relative to normal)

of the normal blood = $\frac{\text{Number of grams of hemoglobin found in 100 cc}}{\text{Normal number of grams of hemoglobin per 100 cc}} \times \frac{\text{Number of cc of packed cells found per 100 cc}}{\text{Normal number of cc of packed cells per 100 cc}}$

$$\frac{15}{15} \times \frac{46}{46} = 1.00$$

of the anemic blood = $\frac{6}{15} \times \frac{184}{460} = 1.00$

$$\frac{6}{15} = 0.4$$

$$\frac{0.4 \times 184}{460} = 1.00$$

The mean corpuscular hemoglobin concentration³ (the concentration of the hemoglobin in per cent per unit volume of cells) is calculated by dividing the number of grams of hemoglobin per 100 c.c. of blood by the number of cubic centimeters of packed cells per 100 c.c.

$$\text{Then the mean corpuscular hemoglobin concentration in the normal blood} = \frac{15}{46} = 32.6$$

$$\text{per cent, in the anemic blood} = \frac{6.0}{18.4} = 32.6 \text{ per cent}$$

The calculation of the different indices is facilitated by the use of a nomogram (Fig. 3)

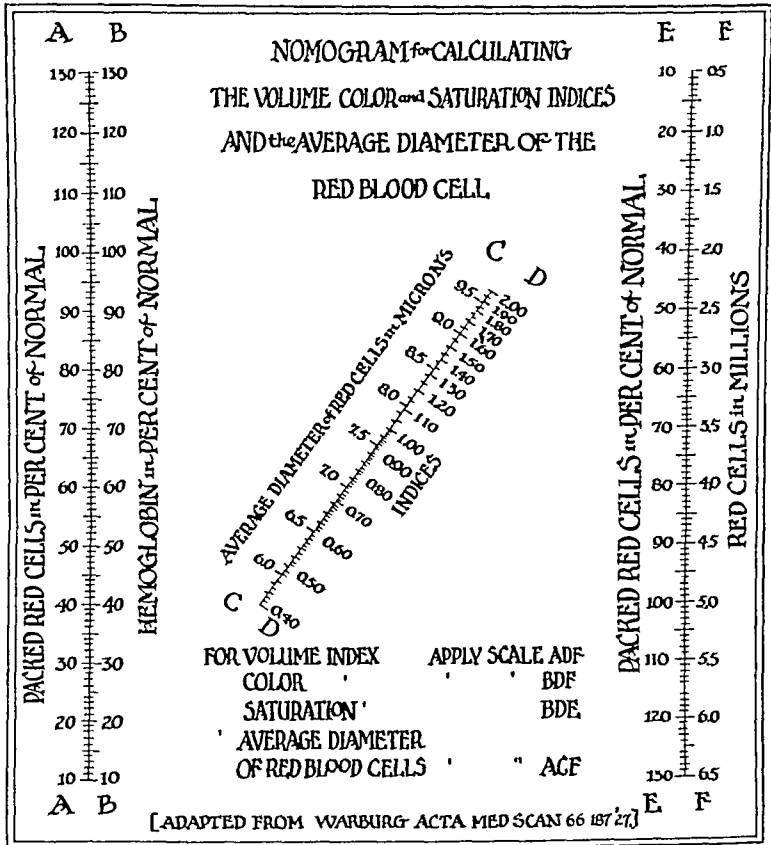


Fig. 3—Nomogram for calculating indices from red cell count hemoglobin in per cent of normal and packed red cells in per cent of normal. The mean diameter of the red blood cells can also be calculated.

5 *White Corpuscle Count*—This is subject to fewer errors and greater variation than is the red cell count but should be equally carefully done.

6 *Preparation of Stained Film*—In many laboratories blood films are made only on slides. For the study of the morphology of the red cells for reticuloocyte counts and for examinations for parasites, such films are satisfactory. For an accurate differential count for determining the relative number of platelets, and for studying the morphology of the white cells, films made on cover glasses are far preferable. The technique of a blood examination is certainly not mastered until one can make satisfactory cover glass preparations. These are easily made

if certain precautions are observed I find no difficulty in having the best of preparations made by efficient technicians

The most satisfactory cover glasses are No 2 $\frac{3}{8}$ inch square of good manufacture They must be absolutely clean and free from dust Some cleaning solution such as a concentrated acid or bichromate acid mixture is often employed The best method of cleaning is to scrub them with some grit-free scouring powder such as Dutch Cleanser The hands are thoroughly washed a number of cover glasses placed in the palm of one and the scouring powder and a

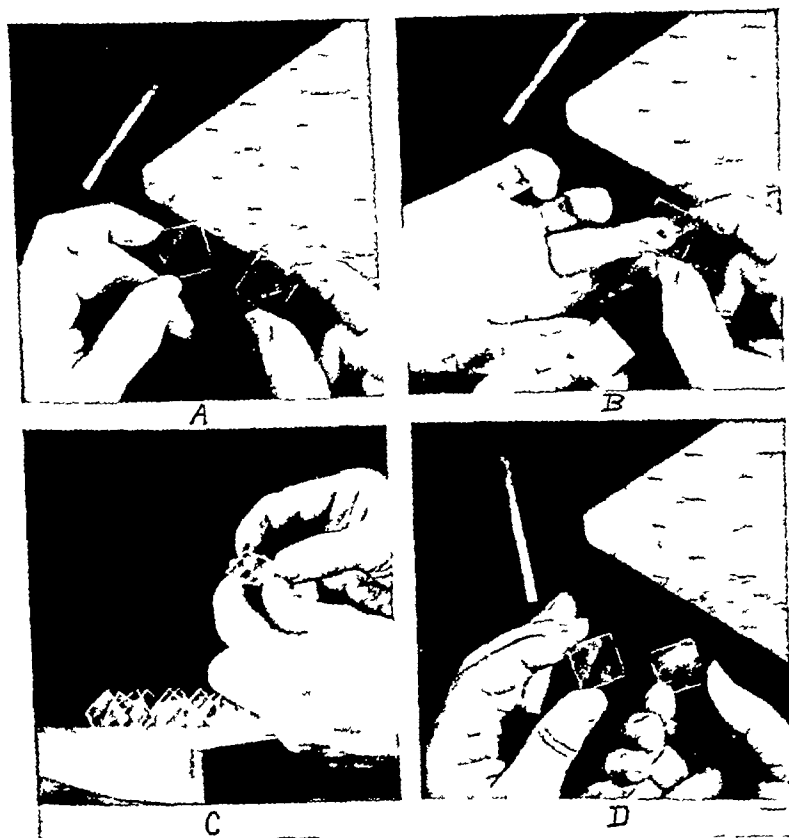


Fig 4—The preparation of blood films by the cover glass method A a cover glass ($\frac{3}{8}$ inch square No 2) is grasped at the adjacent corners with the thumb and forefinger of each hand B the drop of blood is touched with the cover glass held in the right hand C the cover glass carrying the drop of blood is quickly placed parallel on the cover glass held in the left hand D cover glasses are then drawn apart with a sliding motion care being taken to keep them parallel The films are allowed to dry in air and are then ready for staining The drop of blood must be globoid on the finger tip and just large enough to cover the cover glass when properly spread (From Haden—*Clinical Laboratory Methods*)

small amount of water added The glasses are then well scrubbed with the palm of the other hand using a rotary motion They are rinsed with distilled water placed in alcohol dried with a clean lint-free cloth and stored in boxes Just before use they are brushed off with a camel's hair brush and placed on edge in a block of wood or in the top of a box We usually make a number of slits in the top of a 20 cc syringe box and keep in the box an automatic lancet the box of cleaned cover glasses a camel's hair brush cotton gauze and a small bottle of alcohol thus providing everything needed for making blood films

In making the films, a clean and dust-free cover glass is grasped at the adjacent corners with the thumb and forefinger of each hand and the drop of blood on the finger tip is touched with the cover glass held in the right hand (Fig 4). The cover glass carrying the drop of blood is quickly pressed parallel to the cover glass held in the left hand. The blood spreads by capillary attraction. As the spread is completed the cover glasses are drawn apart with a sliding motion, care being taken to keep them parallel. The films are allowed to dry in air and are then ready for staining. The finger is punctured with an automatic lancet since the depth of the puncture wound can be regulated best in this manner. The drop of blood must be globoid on the finger tip and just large enough to cover the cover glass when properly spread.

Staining the Blood Film—The films are best stained on a small stand made by nailing a row of corks to a wood block (Fig 5). Wright's stain is the most satisfactory one for routine use. Only chemically pure, acetone free methyl alcohol such as Merck's Blue Label should be employed in making the staining solution. Cover the blood film with about 10 drops of stain and after one minute add an equal number of drops of distilled water. Very often preparations made in this manner are too blue due to an excess of alkali in the stain. The

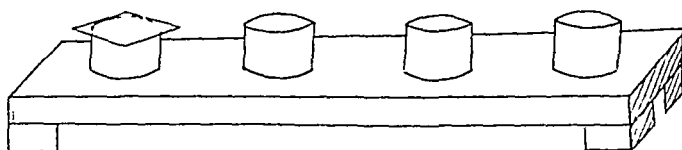


Fig 5.—Convenient stand for staining blood films made on cover glasses (From Haden—*Clinical Laboratory Methods*)

simplest way to correct an excess of alkalinity is by adding a phosphate buffer solution. The optimum amount of buffer solution to be added must be determined by trial. Usually the most satisfactory stains are made by adding 3 drops of a phosphate buffer solution with a P_H equal to 6.4 and 8 to 10 drops of distilled water. If the staining solution is very alkaline, only the buffer solution is used. Let stand for four to five minutes. The phosphate buffer solution with P_H equal to 6.4 is made as follows:

Primary potassium phosphate (KH_2PO_4)	6.63 gm
Anhydrous secondary sodium phosphate (Na_2HPO_4)	2.56 gm
Distilled water to make	1000.00 c c

The stained films are mounted film side down in neutral gum damar solution. A rather thin solution of gum damar in chemically pure xylol is made, calcium carbonate is thoroughly mixed with it and the solution placed in the window in the sunlight for several weeks. After the calcium carbonate has completely settled out, the solution is poured off and placed in a warm place until it has evaporated to the proper consistency. The gum damar thus made is neutral, does not darken with age and does not cause fading of the stain.

7 Count of Reticulocytes—The reticulocytes may be stained with brilliant cresyl blue in a number of different ways. Often a film of cresyl blue is pre-

pared on cover glasses and the blood film made on this. We prefer the following technique. A drop of a saturated solution of brilliant cresyl blue in alcohol is placed on a porcelain drop plate (Fig 6) and allowed to evaporate to dryness. One drop of the blood taken from the centrifuge tube prepared for the determination of the red cell volume is mixed with the stain. This is taken up with a pipette. Films are prepared on cover glasses and counterstained with Wright's stain.

If only a reticulocyte count is to be made a drop of blood from the finger tip is taken up with a capillary, mixed with the dried stain in the drop plate and blood films made from the mixture.

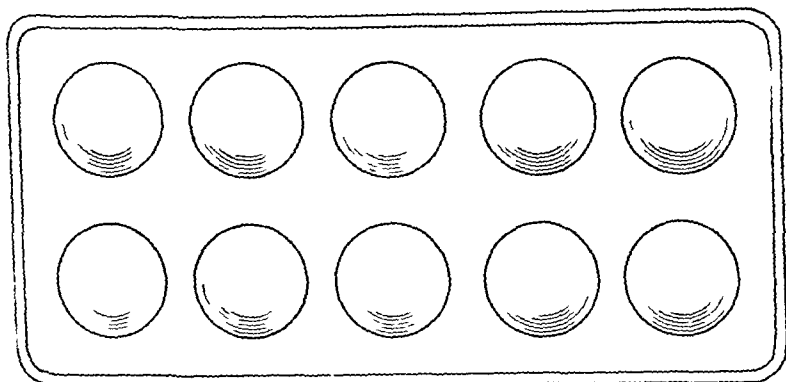


Fig 6—Porcelain mixing plate for use in blood grouping. (From Gradwohl and Blaivas—*Blood and Urine Chemistry*.)

8 *Determinations of Bile Pigment Content of the Blood Plasma*—The bile pigments are easily and satisfactorily estimated as the icterus index. I use the method suggested by Murphy.⁴ For the color comparison a series of standards are prepared from various dilutions made from a 1:100 solution of potassium bichromate to correspond with varying icterus index figures as shown in Table I.

TABLE I

DILUTION	CORRESPONDING ICTERUS INDEX	DILUTION	CORRESPONDING ICTERUS INDEX
1:10,000	1	1:500	20
1:5,000	2	1:400	25
1:2,000	5	1:200	50
1:1,000	10	1:133	75
1:666	15	1:100	100

The solutions are kept in a rack in small test tubes 10 mm. in diameter (Fig 7). One or two cubic centimeters of the supernatant plasma is pipetted from the centrifuge tube after spinning into a similar test tube and compared with the bichromate standards. The figure corresponding to the dilution which matches the serum is the icterus index of the serum. A correction is made for the dilution with oxalate. The normal icterus index is 4 to 6. To avoid clouding blood should be taken when the patient is fasting. In preparing the dilutions of potassium bichromate 2 drops of concentrated sulphuric acid should be added to each 500 cc. to prevent fading.

To Recapitulate—Twenty cubic centimeters of blood have been taken from the patient's vein and blood films have been made from the finger tip. Ten cubic centimeters of blood have been mixed with isotonic sodium oxalate. Before centrifuging films for a count of the reticulocytes have been made from a drop of the oxalated blood. After centrifuging, the volume of red cells has been read off and the icterus index has been determined on the supernatant plasma. Red cell and white cell counts have been made on the specimen to which a drop

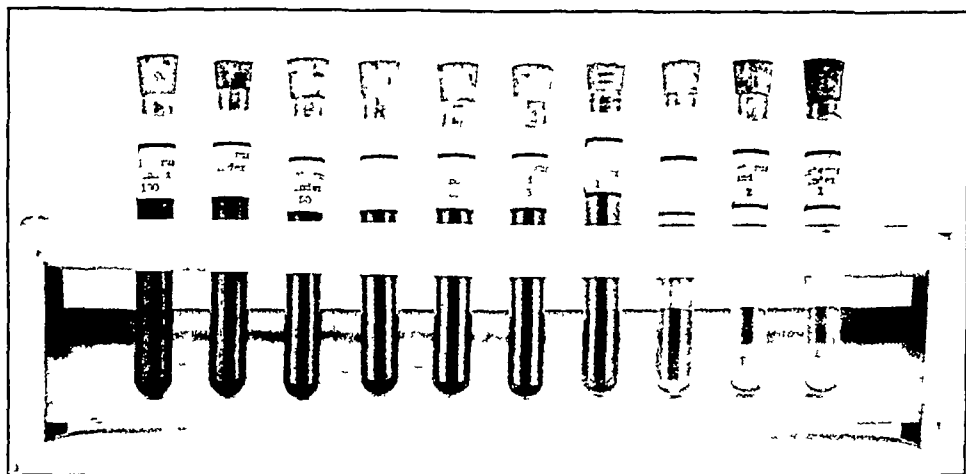


Fig 7—Set of bichromate standards for estimating the icterus index

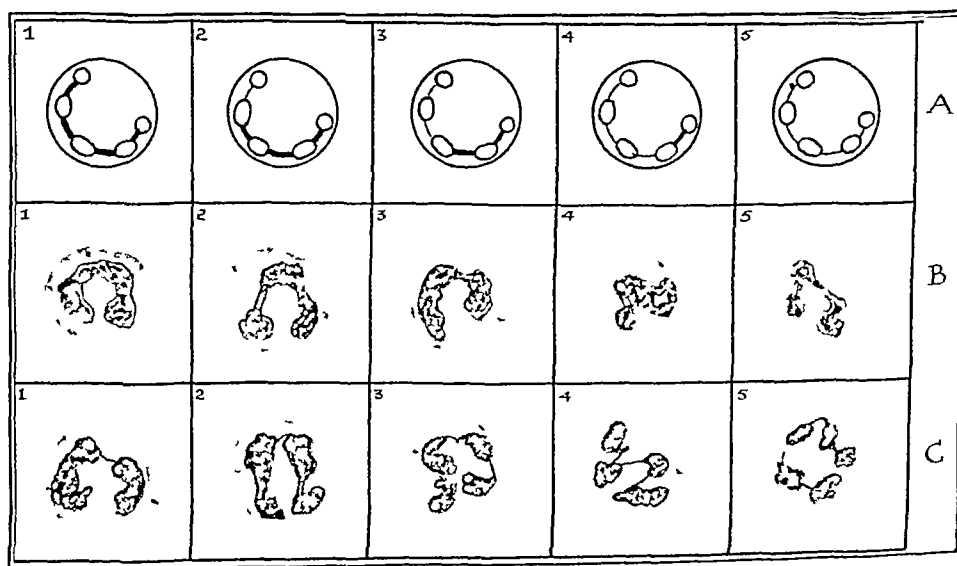


Fig 8—A diagram to illustrate different types of nuclei in polymorphonuclear neutrophilic cells. 1 polymorphonuclear with nucleus of five lobes connected by thick bands of nuclear tissue. The nucleus shows five distinct masses but since the connecting threads are thick the cell is designated nonfilamented. 2 3 4 5 polymorphonuclears in which two or more lobes are connected only by a filament. These four cells are all designated filamented polymorphonuclears. B 1 2 3 4 5 nonfilamented polymorphonuclears. In each cell the lobes of the nucleus are connected by thick threads. C 1 2 3 4 5 filamented polymorphonuclears. In each cell two or more lobes are connected only by a filament of nuclear tissue. (Adapted from Cooke and Ponder—*The Polynuclear Count*.)

of potassium oxalate has been added. The blood film has been stained and examined and the indices have been calculated from the data obtained above. A complete examination has thus been made with a minimum expenditure of time and trouble. Tests other than those outlined above may be indicated. The more common one of definite value is a *special study of white cells*.

a For Maturity. Numerous classifications to indicate the maturity of the polymorphonuclear cells based on a study of the nucleus have been suggested (Arneth, Schilling, Cooke and Ponder, Pons and Kumbhaar). In my opinion the most satisfactory and practical classification is the separation of the polymorphonuclear neutrophils into two groups, filamented and nonfilamented as suggested by Fairley, St. Clair and Reisinger.² Such counts can be made only on well prepared and properly stained blood films on cover glasses. One hundred polymorphonuclear neutrophils are counted. Cells in which the lobes of the nucleus are connected only by a thin strand or filament of nuclear material are counted as filamented cells. If there is any band of nuclear material except this chromatin filament connecting different parts of the nucleus such a cell is counted as nonfilamented (Fig. 8). If 100 polymorphonuclear cells are counted not more than 25 per cent should be nonfilamented. If only 100 white cells of all types are counted not over 16 per cent of the neutrophils should be nonfilamented.

Any irregularity in size and staining reactions of the granules should be noted, since such changes are a good index of the degree of toxicity and may be equally important as variations in maturing of the nucleus.

b For Oxidase Content. This is of value in differentiating cells of the lymphocyte and bone marrow series. I think the best method is a slight modification of the Goodpasture³ stains. The following stock stain is kept on hand:

Alcohol, 95 per cent	100.00 c c
Sodium nitroprusside	0.05 gm
Benzidine c p	0.05 gm
Basic fuchsin	0.05 gm

The sodium nitroprusside is dissolved in 1 to 2 c c of water and added to the alcohol in which the benzidine and fuchsin have been dissolved. Ten drops of the reagent are poured on the blood film, allowed to remain two minutes and then diluted with an equal quantity of phosphate buffer solution ($P_H = 6.4$) containing 0.5 per cent hydrogen peroxide added just before use.

c Jenner-Giemsa Stain for Special Study of Leucocytes. The films stained by Wright's method are satisfactory for most purposes. The Jenner-Giemsa stain brings out beautifully the finer details of nuclear and other cell structures. In leucemia especially such preparations are valuable. They are made as follows:

The cover glass preparation is covered with Jenner's stain for three minutes and an equal number of drops of distilled water added. After one minute the stain is washed off. The cover glass is then placed with the film down in a watch glass. The Giesma stain (15 drops of the stock Giesma solution to 10 c c of distilled water) is run into the watch glass from the side and left for from ten to fifteen minutes. Wash dry and mount in neutral gum damar.

SPECIAL EXAMINATIONS INDICATED IN HEMORRHAGIC DISEASES AND OTHER CONDITIONS

1 *Platelet Count*—An excellent idea of the relative number of platelets may be gained from an examination of a properly made cover glass preparation. If the number seems diminished a count should be done. The Rees Ecker method is a very satisfactory one. A small amount of diluting fluid (sodium citrate 3.8 grams, formalin, 0.2 cc brilliant cresyl blue, 0.1 gram distilled water 100 cc) is drawn into the bulb of the diluting pipette to moisten the capillary. The blood is then drawn up to the 0.5 mark and the bulb filled with the diluting fluid. The counting and calculation is done as for a red cell count.

2 *Determination of Fragility of Erythrocytes*—The method described for this by Giffin and Sanford⁷ is a simple and satisfactory one (Fig. 9). Twelve

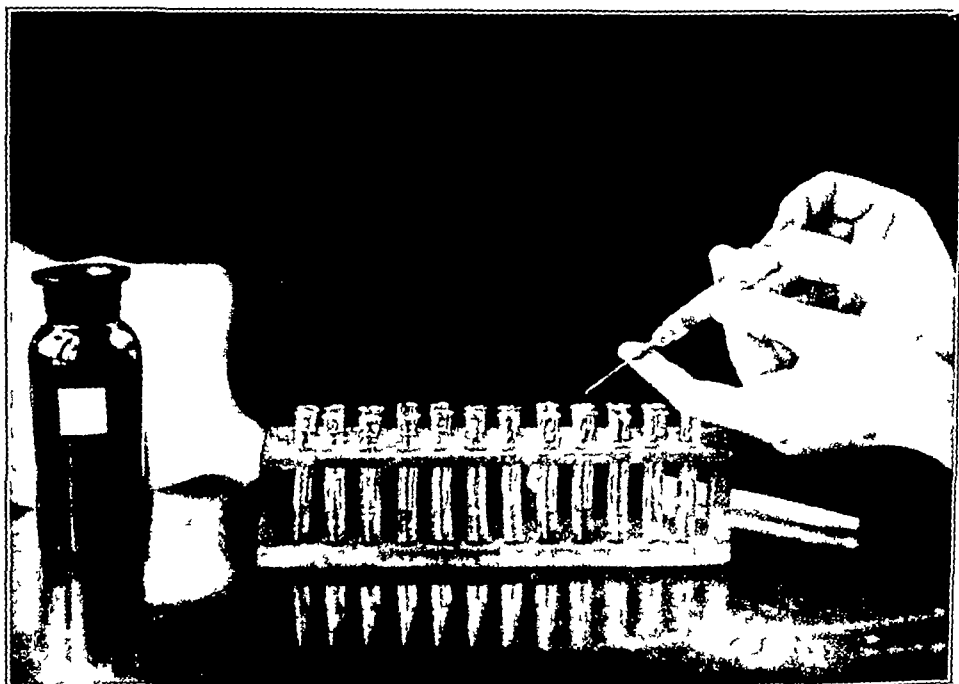


Fig. 9—Method for determination of fragility of red blood cells. One drop of whole blood is added to each tube of hypotonic solution. (After Giffin and Sanford.)

Wassermann tubes are set up in a rack and numbered 25 to 14 from left to right with a capillary pipette run into each tube the number of drops of an accurately made solution of 0.5 per cent sodium chloride being indicated by the figure on the tube. Distilled water is added by means of the same pipette to make the total number of drops of an accurately made solution of 0.5 per cent sodium chloride indicated by the figure on the tube. Distilled water is added with the same pipette to bring the total number of drops in each tube up to 25. Blood is withdrawn from a vein by means of a dry sterile syringe and one drop run into each tube. The tubes are allowed to stand at room temperature for one hour or more. The dilution in which there is just a slight tingeing of the supernatant fluid due to laking of a few of the least resistant corpuscles is noted as the point of initial hemolysis. Reading from left to right complete hemolysis

is indicated in the first tube in which no corpuscular residue is evident by shaking the tube

The percentage of sodium chloride in any tube is calculated by multiplying the number on the tube by 0.02. Normal blood shows intense hemolysis in 0.42 or 0.38 per cent sodium chloride solution and complete hemolysis in 0.36 to 0.32 per cent

3 Determination of Coagulation Time—It is a waste of time to determine the coagulation time on a drop of blood obtained by piercing the skin. The method suggested by Lee and White⁵ is a satisfactory one for clinical use. Blood is withdrawn from a vein with a syringe in which the space between the end of the plunger and the needle is filled with salt solution and one cubic centimeter is run into each of 3 small Wassermann tubes 8 mm in diameter. The tubes should be scrupulously clean and washed with salt solution just before use. After standing for three minutes a tube is rotated endwise every thirty seconds and that point at which the blood no longer flows from its position but maintains its surface contour when inverted is taken as the end point. Normal blood coagulates by this method in five to eight minutes.

4 Bleeding Time—This is easily and quickly done by the method of Duke.⁶

The blood report form is a convenient one on which to report the results of the examination.

BLOOD REPORT

Name	Date
Case No.	Service

1 RED BLOOD CELLS

1 Number per c mm—

2 Size in stained preparation—

3 Shape in stained preparation—

4 Color in stained preparation—

5 Regeneration forms

(a) Nucleated red cells—

(b) Basophils punctate or diffuse—

(c) Nuclear particles—

6 Fragility hemolysis begins in % complete in %
sodium chloride (normal)

7 Reticulocyte count—

2 VOLUME OF PACKED RED BLOOD CELLS % of normal (cc per 100 cc)
3 VOLUME INDEX (Mean corpuscular volume = cubic microns)

4 HEMOGLOBIN % of normal (gm per 100 cc with hemoglobinometer)

5 COLOR INDEX (Mean corpuscular hemoglobin = micromicrograms)

6 SATURATION INDEX (Mean corpuscular hemoglobin concentration = per cent)

7 WHITE BLOOD CELLS

1 Number per c mm—

2 Differential count

Neutrophils—	%	Eosinophiles—	%	Basophiles—	%
Lymphocytes—	%	Monoocytes—	%		
Nonfilamented neutrophils—			% (normal 6-16%)		

3 Presence of abnormal forms

(a) Myelocytes—

(b) Myeloblasts—

(c) Lymphoblasts

(d) Fragile leucocytes

(e) Toxic neutrophils

8 PILE PIGMENTS IN PLASMA

(a) Icterus index

(b) Units (van den Bergh) per 100 cc (Normal 4 to 6)

9 PLATELETS per c mm (Normal 0.5 to 2 units)

10 COAGULATION TIME (method)

11 REMARKS

12 LABORATORY DIAGNOSIS

Name of Examiner _____

THE LABORATORY CLASSIFICATION OF ANEMIA ON THE BASIS OF VOLUME AND HEMOGLOBIN CONTENT

The laboratory classification of anemia has always been unsatisfactory. A rough differentiation into primary and secondary types is very frequently used. The anemias having a color index greater than 1.00 are usually classified as primary and those having a color index of 1.00 or less as secondary. Hampson and Shackle¹⁰ first suggested the classification of anemias on the basis of cell size, using the terms "megaloeytic" and "nonmegaloeytic." Wintrobe¹¹ suggested four groups: (1) macroeytic, (2) normoeytic, (3) simple microeytic and (4) hypochromic. Certainly the most logical laboratory classification is based on all three variants of the erythrocyte, namely, number, size, and hemoglobin content. The following terms may well be employed to indicate variations which have been observed in these factors:

Number	$\left\{ \begin{array}{l} \text{Hypercythemie} = \text{red cell count} > \text{normal} \\ \text{Normocythemie} = \text{red cell count within normal limits} \\ \text{Hypocythemie} = \text{red cell count} < \text{normal} \end{array} \right.$
Volume	$\left\{ \begin{array}{l} \text{Macroeytic} = \text{mean corpuscular volume} > \text{normal (VI} > 1.10) \\ \text{Normoeytic} = \text{mean corpuscular volume} = \text{normal (VI} = 0.90-1.10) \\ \text{Microeytic} = \text{mean corpuscular volume} < \text{normal (VI} < 0.90) \end{array} \right.$
Hemoglobin Content	$\left\{ \begin{array}{l} \text{Hyperchromic} = \text{Mean corpuscular hemoglobin} > \text{normal (C.I.} > 1.10) \\ \text{Normochromic} = \text{Mean corpuscular hemoglobin} = \text{normal (C.I.} = 0.90-1.10) \\ \text{Hypochromic} = \text{Mean corpuscular hemoglobin} < \text{normal (C.I.} < 0.90) \end{array} \right.$

All the different types of anemia which may occur from this standpoint are

Normocythemie	$\left\{ \begin{array}{l} \text{Normoeytic and hypochromic} \\ \text{Microeytic and hypochromic} \end{array} \right.$
Hypercythemie	$\left\{ \begin{array}{l} \text{Normoeytic and hyperchromic} \\ \text{Microeytic and hyperchromic} \end{array} \right.$
Hypocythemie	$\left\{ \begin{array}{l} \text{Macroeytic and hyperchromic} \\ \text{Macroeytic and normochromic} \\ \text{Macroeytic and hypochromic} \\ \text{Normoeytic and normochromic} \\ \text{Normoeytic and hypochromic} \\ \text{Microeytic and hypochromic} \end{array} \right.$

These different types of anemia are illustrated in Chart 1. The circles indicate relative volume, not diameter, and the intensity of color indicates the relative hemoglobin content. A typical example of each type of anemia is given. Every anemia should be thought of in terms of number, volume and hemoglobin content of the average erythrocyte, and every case should be classified on such criteria. An anemia with a red cell count of 3,500,000, a volume index of 0.75 and a color index of 0.65 is reported as a hypocythemie microeytic and hypochromic anemia rather than simply as "secondary" anemia. Likewise an anemia with a count of 2,000,000 and a volume and color index of 1.50 is recorded as hypercythemie macroeytic and hyperchromic rather than "primary."

SUMMARY

I have tried to emphasize the need for an accurate and complete examination of the blood in studying hematologic problems. Any clinician who has had the opportunity of utilizing such an examination will never be satisfied with any other kind. A complete blood study may be made quickly and simply in a well equipped laboratory.

I have indicated one satisfactory technique for each of the tests suggested, although others may be equally satisfactory. One good method should be used until it is thoroughly mastered.

Only with such laboratory data can an accurate knowledge of the blood dyscrasias be gained.

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THE VALUE OF ACCURATELY DETERMINED COLOR, VOLUME AND SATURATION INDEXES IN ANEMIAS¹

BASED ON A STUDY OF OVER TWO HUNDRED PATIENTS

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RECENT developments¹ in the therapy of anemias have greatly increased the importance of accurate differential diagnosis. The value of the color index in differentiating between pernicious and other anemias was learned long ago, but the use of inaccurate methods and incorrect standards for calculation have prevented the full realization of its possibilities. The more recently introduced volume and saturation indexes have been far less thoroughly studied, but appear to have equal or greater diagnostic value.

The perfecting of a simple hemoglobin method^{2, 3} of research accuracy, a uniform system of hematologic methods⁴ for use with oxalated venous blood, and the establishment of normal standards^{5, 6, 7, 8} for comparison has made possible the present restudy of the value of these indexes in the differential diagnosis of anemias. This study has revealed far more diagnostic value for these indexes than we had dared to hope.

REVIEW OF THE LITERATURE

Apparently Johannes Duncanson (1867) was the first to recognize the possibility of variation in size and in hemoglobin content of the red cells in different diseases, thus forming a clear concept of the information which the color, volume and saturation indexes give although he did not actually determine them. He did demonstrate the relative decrease in hemoglobin content and size of the red cells in chlorosis.

The color index was first calculated by Havem^{10, 11} (1878). Leeuwenhoek¹² (1673) was apparently the first to measure the size of red cells by their diameter, but Welcker¹³ (1864) was the first to make clinical application of the method. He demonstrated that the blood corpuscles in chlorosis were smaller than normal.

Sørensen¹⁴ (1876) was apparently the first to point out the fact that the increase in size of the red cells is one of the most characteristic features of pernicious anemia, but the popular spread of this knowledge was due largely to Paul Ehrlich¹⁵ (1880) and to Litcher¹⁶ (1883). Bleibtren and Bleibtren¹⁷ (1892) were the first to work out a clinically practical method for determining the red cell volume, but did not apply it to hematology. Credit for the introduction of the hematocrit for the determination of red cell volume belongs to Hedin¹⁸ (1890) and Blix, Daland²⁰ (1891), and Gaertner²¹ (1892). Herz²² (1893) was the first to use a quantitative expression for the ratio of hemoglobin to cell volume which he called the "spezifische Hämoglobingehalt". He divided the hemoglobin estimation by the total cell volume, thus securing a figure corresponding to the hemoglobin index of Whipple and Robscheit Robbins²⁶. He had a full understanding of the various

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²Presented before the Pacific Interurban Clinical Club July 5 1929 and demonstrated at the Scientific Exhibit of the American Medical Association July 1929 at Portland Oregon.

possible relationships between cell count, volume, and hemoglobin content. He reports results on several cases.

By the end of the nineteenth century the color index was in common use and findings in different types of anemia had been fairly thoroughly studied. Methods were available for the determination of cell volume and diameter and a few students of the anemias knew of the more important changes in cell size and hemoglobin content which occur. The volume index and saturation index had not been calculated and very few individuals had any clear concept of the changes in the red cells which these indexes express. Full reviews of this early literature will be found in the monographs of Eichhorst,¹⁰ Laache,¹¹ Reinert,²² and in the writings of Ehrlich, Lazarus, and Negehn.²⁴

In 1901, Capps²⁵ first reported the method for calculation of the volume index and in 1903,²⁶ he reported detailed studies on the blood of 175 persons, 10 of whom were normal. These studies included hemoglobin estimations by the Fleischl method, red cell counts, cell volume determinations by the hematocrit* method, computation of the average diameter of the red cells based on the measurement of 100 cells with the eyepiece micrometer, and the calculation of the color and the volume index. Had his methods been as accurate as those of today, undoubtedly he would have discovered almost everything that is now known of the value of these indexes. The failure to recognize the importance of this work has postponed for at least twenty five years the attainment of the accuracy in diagnosis of anemias which a full understanding of his researches makes possible. Notwithstanding the fact that with the technique used he could not have obtained full packing of the red cells, that his hemoglobin estimations cannot be transformed into absolute values, and that he based his normal standards for comparison on the study of only four men and six women, he arrived at the following conclusions, all of which have since been confirmed:

"1 The centrifuge accurately determines the mass of red corpuscles, but cannot be relied upon to estimate the number of cells, because the volume of the cell undergoes variations in disease.

"2 The volume of the individual erythrocyte is best obtained by using the centrifuge in conjunction with the hemacytometer. Volume Index is an expression used to designate the volume of the erythrocyte relative to the normal. Measurement of diameters for the determination of cell size is of limited value and often misleading, especially when poikilocytosis is present.

"3 The cell volume is invariably increased in pernicious anemia and usually more so than the Hb content of the cell. This heightened volume index is a more constant and trustworthy sign of pernicious anemia than the increased color index. The polychromemia in pernicious anemia is due to an increase in cell volume and not to an increased affinity of the protoplasm for Hb.

"5 In a large proportion of chlorotics the cell volume suffers as well as the Hb, although always to a less extent. The volume index is of great significance in prognosis.

"7 The Hb content of a normal erythrocyte, as indicated by a color and volume index of 1.00, represents the point of saturation of the protoplasm. When, therefore, the color index rises above 1.00, we assume that a corresponding increase has taken place in the cell volume (except in jaundice, where the color test is unreliable). Supersaturation of cell protoplasm with Hb probably does not occur. On the other hand, the cell may lose Hb without necessarily losing in volume.

"8 Cell volume seems to be chiefly altered by influences affecting cell growth or degeneration. The large erythrocytes of pernicious anemia are probably young cells. Small cells may result from a malnutrition of the bone marrow, as in chlorosis, or from an actual degeneration, as in sepsis.

"9 The cell volume suffers remarkably little change from osmotic influences according to my observations. Dropsy, cyanosis, the hydrema following acute hemorrhage, and jaundice (with some exceptions) do not materially alter the volume of the cells."

His data permit many conclusions which he did not report.

*He centrifugated at 10 000 revolutions per minute for three minutes relying only on speed of manipulation to secure packing of the cells before clotting occurred.

Wroth²⁸ (1907) studied eleven cases of anemia, but added nothing to the work of Capps. In 1911, Larrabee²⁹ studied 139 individuals, 21 of whom were normal, but his methods were so inaccurate that his results and conclusions are incorrect in many cases. Alder³⁰ (1918), using a viscosimetric method for the determination of cell volume, studied eleven cases of anemia. He was apparently the first after Herz²⁷ to use a quantitative method for expressing the relationship of the hemoglobin to the cell volume, the ratio now expressed by the saturation index. He simply divided the hemoglobin percentage by the cell volume per 100 c.c., considering 2.2 to 2.4 as the limits of normal variation. This ratio corresponds to the hemoglobin index of Whipple and Robschert Robbins.³⁰ Alder was the first to state that red cells tend to approach the spherical form in hemolytic icterus. He confirmed Capps's observation that the concentration of hemoglobin in the red cell is not increased in pernicious anemia, but that the increased color index is due to the increased average cell volume and that the hemoglobin content of the red cell is low in chlorosis.

Bonninger³¹ (1919) was the first to calculate the saturation index in essentially its present form. He called it the *Farbeindex Volumen* in contradistinction to the color index which he called *Farbeindex Zahl*. He gave cell volume figures in terms of cubic micra of the average red cell instead of in terms of the volume index, but the latter may readily be calculated from his data. In a previous paper, he studied results on sixty normal persons and he adds twenty in this paper. He was thus the first to study any adequate series of normals. His red cell counts and hemoglobin estimations are obviously inaccurate, but his cell volume determinations were much better than any previously made. His average figures for total volume of packed red cells per 100 c.c. of blood, namely, 44.7 c.c. for men and 41.0 c.c. for women, would stand today. He reports results on 61 patients with various diseases, being the first following Capps to give data of clinical value on an extensive series of patients. He confirmed most of the statements of Capps and Alder.

Gram³² (1920) reported very briefly his conclusions from volume index studies on 611 persons.

Suzuki³³ (1920) and Reich³⁴ (1921) review and compare methods for the determination of cell volume. Reich was also apparently the first to use oxalated blood for hematologic study. He reports results, including color and saturation indexes (*Farbeindex Volumen*), on 33 patients with various diseases, 15 of whom he inadvisedly used as normals. His methods were so inaccurate, however, that his figures have very little value.

Csaki³⁵ (1922, claims work was completed in 1919) was the first to emphasize centrifugation to a constant volume rather than for a definite time interval. He calculated color, volume and saturation indexes (*Farbeindex Volumen*). Had he had an adequate series of strict normals for comparison, his results would have been more accurate than those of any of his predecessors. Unfortunately, he based his normals on the study of 23 hospital patients. He reports results on 47 patients with various diseases and confirms most of the points mentioned by Capps²⁶ and Bonninger³¹. Many of his conclusions do not seem warranted by the data presented and have not been borne out by subsequent investigations.

Carrie Froehlich³⁶ (1922) reviewed the literature and studied 95 healthy persons and 64 patients. She published results of color indexes on 65 normals and 28 patients and results of color and saturation indexes (*Farbeindex Volumen*) together with the average volume of one red cell on an additional 20 normals and 36 patients. Her technique, calculations, and method of selecting normals were far more satisfactory than those of any of her predecessors. Her figures are the first to show that the red cell is slightly larger in women (by 1.3 per cent) than in men.

Rosendale³⁷ (1923) reports results of studies on a few patients using unsatisfactory methods. His conclusions are difficult to correlate with his data.

In 1923 while the present study was in progress Haden's³⁸ work appeared. This study was based on 40 normal men, 12 normal women, 20 cases of pernicious anemia, and 32 patients which he classed as having secondary anemia. His determinations were all done on oxalated venous blood and the hemoglobin estimations were far more accurate than those of his predecessors. However, he neglected to centrifuge to constant volume, and it has since been shown that the time and speed of centrifugation which he used are not sufficient

for complete packing of the red cells in all cases. Apparently unaware of the previous work on the saturation index (Tarbindex Volumen), he coins this term and gives directions for calculating this index, which Capps³⁸ had so nearly discovered (see paragraph 7 of his conclusions quoted above). Most of the current interest in the color, volume and saturation indexes dates from this article by Haden.

Drucker³⁹ (1924) studied the hemoglobin, cell volume, and saturation of the cell with hemoglobin in 270 healthy children and 217 children with various diseases. Unfortunately, he did not do red cell counts.

In 1924, Haden⁴⁰ republished his previous results with the addition of 30 cases of pernicious anemia and 20 of secondary anemia.

In 1926, Osgood⁴¹ suggested a tentative classification of anemias by color, volume and saturation indexes, based on the study of the first 63 cases included in the present paper. In this classification, the diagnostic value of a low saturation index in anemias of chronic blood loss was first pointed out.

Jørgensen and Warburg⁴² (1926) give a very valuable review of the literature on the size and hemoglobin content of the red cell. They report color, volume and saturation indexes with measurement of cell diameter on 7 normal persons and 45 patients. The most complete bibliography of this subject yet published is appended.

TABLE I

	RED CELLS MILLION PER C MM			HEMOGLOBIN GRAMS PER 100 CC			CELL-VOLUME CC PACKED CELLS PER 100 CC OF BLOOD		
	AVER AGE	RANGE	RESULTS IN 90 PER CENT OF THE CASES	AVER AGE	RANGE	RESULTS IN 90 PER CENT OF THE CASES	AVER AGE	RANGE	RESULTS IN 90 PER CENT OF THE CASES
Men (196)	54	4.46 to 6.40	4.70 to 6.10	15.80	13.44 to 20.11	14.00 to 18.00	44.79	36.09 to 51.87	40.10 to 48.97
Women (106)	48	4.14 to 5.55	4.30 to 5.30	13.70	10.98 to 16.49	12.00 to 15.50	41.00	35.32 to 45.89	37.20 to 45.00

Greppi⁴² (1927) reports color and volume indexes, together with total blood and plasma volume determinations, on 6 patients with pernicious anemia and 12 patients with secondary anemia.

Cameron⁴³ (1928) reports color and volume indexes and grams of hemoglobin per 100 c.c. of packed cells on 10 normal persons, 10 cases of pernicious anemia, and 25 other patients, drawing the conclusion that "in pernicious anemia the hemoglobin content of a given volume of red blood corpuscles is invariably above the average normal value, and usually much above this value." This statement is directly contrary to the statement of Capps, Haden, and others that supersaturation of the cell with hemoglobin does not occur.

Some time after the first presentation⁴⁴ (July, 1929) of the data and conclusions included in this paper, Wintrobe⁴⁵⁻⁴⁶ (May, 1930) published preliminary reports of studies on 140 patients with anemia. He⁴⁵ suggests a classification very similar to that previously suggested by one of us⁵ (1926).

SELECTION OF SUBJECTS

More than 200 patients* were selected for study because they had been diagnosed as anemic by some one. Time did not permit the study of all such patients available during the seven years over which this research extended,

*These were from the Multnomah County Hospital and from the private practices of Drs Harold C. Bean, I. C. Brill, N. W. Jones, Laurence Selling, and others.

so our figures have no value for a determination of the relative frequencies of different types of anemia

All cases were discarded from this series in which an almost certainly complete and correct diagnosis was not *ultimately* made by criteria other than the color, volume and saturation indexes and agreed to by other physicians seeing the case. This left a group of 167 examinations on 144 cases which will be discussed in detail

METHODS*

The clinical examinations were carefully checked by one of the authors. Oxalated venous blood was used for the hematologic methods.⁴ Gastric contents analyses, stool examinations and urinalyses including tests for urobilinogen, were done in almost all cases.

Red cell counts were made with research accuracy, using Toisson's diluting fluid and Bureau of Standards apparatus. The counts reported are the

NORMALS

COEFFICIENTS		INDEXES								
HEMOGLOBIN	VOLUME	COLOR			VOLUME			SATURATION		
AVER. AGE	AVER. AGE	AVER. AGE	RANGE	RESULTS IN 90 PER CENT OF THE CASES	AVER. AGE	RANGE	RESULTS IN 90 PER CENT OF THE CASES	AVER. AGE	RANGE	RESULTS IN 90 PER CENT OF THE CASES
14.66	40.70	1.00	0.84 to 1.21	0.90 to 1.12	1.00	0.78 to 1.12	0.90 to 1.08	1.00	0.90 to 1.23	0.90 to 1.10
14.34	42.83	1.01	0.83 to 1.19	0.90 to 1.10	1.00	0.86 to 1.11	0.90 to 1.10	1.00	0.87 to 1.16	0.90 to 1.10

average of two or more dilutions agreeing within 100,000. Hemoglobin estimations were made by the Osgood-Haskins method.^{2,3} Cell volume determinations** were made by the method⁵ previously described by us. Centrifugation was carried out until the cell volume remained constant, a precaution which has been neglected by many other workers. A correction of 3.5 per cent of the volume of the packed red cells must be added if it is desired to compare the results with those in which an isotonic anticoagulant was used. Control study showed each of these three methods to have limits of error of plus or minus 2 per cent.

Calculation of Indices—The normal standards (Table I) used were determined by the authors on 196† healthy young men and 106† healthy young

* All details of the methods used may be found by reference to the *Textbook of Laboratory Diagnosis* by Edwin L. Osgood and Howard D. Haskins. P. Blakiston's Son & Co., Inc., Philadelphia.

** We consider that Ponder's⁶ study of our method of determining cell volume is a confirmation of its accuracy as his average on ten cases differs from our average on 153 cases by less than twice the probable error of his results.

† Hadcroft in his recent excellent review of red cell volume determinations in man now recognizes the importance of centrifugation to constant volume but criticizes the use of a hypertonic anticoagulant. The justice of this criticism is recognized but we think that the advantages far outweigh the disadvantages.

‡ Since the original papers were published hematologic studies of fifty-nine healthy men and six healthy women have been added to the series without significantly changing any of the figures reported.

women,⁶ using the same technic and apparatus as that used in the present research. Our standards have been shown^{7,8} to agree closely with the averages of all reliable studies made to date.

The indexes were calculated as follows:

$$\text{Color Index} = \frac{\text{Per cent Hemoglobin, when the hemoglobin coefficient*}}{\text{Per cent Red Cells}}$$

for normal persons (for men, 147, for women, 143) of the patient's sex and age group is taken as 100 per cent hemoglobin and when 5 million per cmm is taken as 100 per cent red cells. It expresses the ratio of the hemoglobin per unit number of cells in the patient's blood to the average hemoglobin per unit number of cells in the blood of normal persons of the patient's sex and age group.

$$\text{Volume Index} = \frac{\text{Per cent Cell Volume, when the volume coefficient**}}{\text{Per cent Red Cells}}$$

for normal persons (for men, 41, for women, 43) of the patient's sex and age group is taken as 100 per cent cell volume and when 5 million per cmm is taken as 100 per cent red cells. It expresses the ratio of the mean size of the cells in the blood examined to the mean size of the cells in the average blood of normal individuals of the patient's sex and age group.

$$\text{Saturation Index} = \frac{\text{Per cent Hemoglobin, when the hemoglobin coefficient and the volume coefficient for normal persons of the patient's sex and age group are taken as 100 per cent hemoglobin and cell volume, respectively. It expresses the ratio between the hemoglobin per unit volume of cells in the blood examined and the average hemoglobin per unit volume of cells in the blood of healthy persons of the same sex and age group.}}{\text{Per cent Volume}}$$

In the present study all calculations were made with five place logarithms, but for clinical use a table and a chart¹⁰ have been prepared which greatly simplify these calculations.

A smear stained with Wright's stain was studied and saved for reference in each case. Other hematologic studies such as white and differential cell counts, icterus index determinations, platelet and reticulocyte counts, fragility tests, peroxidase stains, etc., were made as indicated. Whenever possible, an autopsy was performed on those patients who died.

RESULTS

Untreated Pernicious Anemia—In Table II are shown the results of 43 blood examinations on 37 cases (20 men and 17 women) of untreated pernicious anemia. Patients 3 and 10 were unusually young for pernicious anemia, but in Case 3 the diagnosis was confirmed by necropsy and in Case 10 the achlorhydria, combined system disease, leucopenia, and normal red cell fragility seemed to us sufficient evidence to exclude the former diagnosis of familial hemolytic icterus and to establish the diagnosis of pernicious anemia. This patient died, but no necropsy was secured.

A therapeutic test was not applied in most cases because they were studied

*The *hemoglobin coefficient* is a term introduced by the authors for the grams of hemoglobin per 100 c.c. of blood calculated to a red cell count of 5 million per cmm.
 **The *volume coefficient* is a term introduced by the authors for the volume of packed red cells per 100 c.c. of blood calculated to a red cell count of 5 million per cmm.

before the discovery of "live" (nuclear) therapy. Free hydrochloric acid was not found in the stomach contents of any case reported as pernicious anemia.

Note in Table II the uniformly high color and volume indexes with normal saturation indexes. This completely confirms the statement of Capps²⁶ Bonninger³¹ Haden³⁵ and others that the fundamental alteration in the red cell in pernicious anemia is an increase in size with a corresponding increase in hemoglobin content. In no case is there a true hyperchromia as is claimed by Cameron⁴³ and as is stated in most of the older texts.

TABLE II
UNTREATED PERNICIOUS ANEMIA

CASE NO.	SEX	AGE	RED CELL COUNT MILLIONS PER C. MM.	HEMOGLOBIN PER CENT**	GRAMS PER 100 C.C.	VOLUME OF CELLS PER 100 C.C.	COLOR INDEX	VOLUME INDEX	SATURATION INDEX
1	M	52	0.51	16.6	2.29	7.00	1.54	1.69	0.91
2	M	38	0.42	16.7	2.31	7.14	1.89	2.09	0.90
*3	M	24	0.53	16.8	2.32	6.58	1.50	1.53	0.98
4	F	60	0.64	18.5	2.55	7.18	1.39	1.31	1.07
5	M	57	0.53	18.5	2.55	7.79	1.64	1.79	0.91
6	F	65	0.45	18.9	2.61	9.35	2.04	2.43	0.84
			1.21	39.9	5.51	18.75	1.59	1.80	0.88
*7	F	49	0.64	20.9	2.88	7.56	1.59	1.38	1.15
8	M	66	0.95	28.6	3.94	10.91	1.41	1.40	1.01
9	F	68	1.01	29.5	4.07	11.34	1.41	1.31	1.08
10	F	16	1.02	34.7	4.79	14.33	1.64	1.63	1.01
11	F	77	0.80	30.1	4.15	10.27	1.82	1.50	1.22
12	M	62	1.02	37.2	5.14	13.00	1.71	1.55	1.10
13	F	71	1.14	38.8	5.35	13.11	1.64	1.33	1.23
14	F	59	1.18	40.2	5.55	14.84	1.64	1.46	1.12
15	M	44	1.33	40.7	5.62	15.36	1.44	1.41	1.02
16	F	72	1.39	41.9	5.79	15.89	1.45	1.33	1.09
17	M	75	1.28	42.8	5.90	18.39	1.57	1.75	0.90
18	M	74	1.26	43.5	6.00	18.67	1.62	1.81	0.90
19	F	71	1.63	43.9	6.06	18.27	1.30	1.30	1.00
20	M	65	1.32	44.4	6.13	16.00	1.58	1.48	1.07
21	M	78	1.51	46.4	6.41	17.25	1.44	1.39	1.04
22	M	62	1.64	48.0	6.62	17.48	1.43	1.30	1.10
*23	F	50	1.77	50.7	7.00	19.58	1.38	1.29	1.07
24	F	57	1.62	52.0	7.18	18.89	1.55	1.36	1.14
25	M	57	1.99	55.5	7.66	20.81	1.31	1.28	1.03
			1.32	38.9	5.36	14.95	1.38	1.38	1.00
26	M	55	1.92	60.3	8.31	19.28	1.47	1.22	1.20
27	F	66	2.13	61.4	8.47	26.89	1.39	1.47	0.95
			2.09	64.1	8.85	25.71	1.48	1.43	1.03
28	M	61	2.66	63.3	8.73	27.52	1.12	1.26	0.88
29	M	56	2.40	64.9	8.95	26.84	1.24	1.36	0.91
30	F	67	2.28	66.5	9.18	27.69	1.41	1.41	1.00
31	F	66	2.76	72.0	9.94	32.50	1.26	1.37	0.92
			1.28	36.3	5.01	13.23	1.37	1.20	1.14
32	M	45	2.53	74.0	10.21	32.98	1.37	1.59	0.86
33	F	51	2.43	79.5	10.97	32.10	1.58	1.54	1.03
			2.58	80.7	11.14	32.79	1.51	1.48	1.02
34	M	67	2.91	82.0	11.32	33.16	1.32	1.39	0.95
35	F	70	3.21	84.2	11.62	32.23	1.27	1.17	1.08
36	M	66	3.06	88.0	12.14	36.21	1.35	1.44	0.94
37	M	49	3.71	88.7	12.24	39.73	1.12	1.31	0.86
			2.87	81.8	11.29	32.83	1.35	1.40	0.96
Minimum		16	0.42	16.6	2.29	6.58	1.12	1.17	0.84
Maximum		78	3.71	88.7	12.24	39.73	2.04	2.43	1.23
Average		59	1.65	48.9	6.75	19.59	1.48	1.47	0.99

Diagnosis confirmed by necropsy.

**100 per cent hemoglobin is equivalent to 13.5 grams per 100 c.c. of blood.

Observe that 100 per cent of the diagnoses would have been correctly made if both the color and volume indexes were determined and if a result of over 1.25 in either were considered diagnostic of pernicious anemia. Only one case (35) would have been missed if the volume index alone had been used, and only two (28 and 37) if an accurate color index alone had been relied on. These facts should be kept in mind for comparison with the clinical diagnostic average on first examination which will be discussed later. The average color index is 1.48 with a range of 1.12 to 2.04. The average volume index is 1.47 with a range of 1.17 to 2.43. The average saturation index is 0.99, or almost exactly normal, and the range is 0.84 to 1.23 which is almost identical with the normal range as shown in Table I.

Study of the stained smears in some of these cases showed very few deviations from the normal, although it is possible that the tedious procedure of measuring the diameters of 1000 cells might have led to the diagnosis. Megaloblasts and nucleated red cells were found so inconstantly that it was evident that failure to find them cannot be used as evidence against the diagnosis of pernicious anemia. On the other hand, typical megaloblasts were found in cases of leukemia, lead poisoning and carcinoma with metastases to the bone marrow, hence they are far from conclusive proof of the presence of pernicious anemia even when found.

It is noteworthy that in the cases (34 to 37) in which the hemoglobin is comparatively high the color and volume indexes are still sufficiently high to be of diagnostic value.

TABLE III
TREATED PERNICIOUS ANEMIA

CASE NO.	SEX	AGE	DAYS OF TREATMENT	RED CELL COUNT MILLIONS PER C. M.	HEMOGLOBIN PER CENT	GRAMS PER 100 C. C.	VOLUME OF CELLS PER 100 C. C.	COLOR INDEX	VOLUME INDEX	SATURATION INDEX
*11	F	77		0.80	30.1	4.15	10.27	1.82	1.50	1.22
			67	3.61	77.5	10.70	34.13	1.04	1.10	0.94
*33	F	51		2.43	79.5	10.97	32.10	1.58	1.54	1.03
			?	4.02	95.1	13.12	41.04	1.14	1.19	0.96
*34	M	67		2.91	82.0	11.32	33.16	1.32	1.39	0.95
			10	3.32	90.0	12.42	31.52	1.27	1.16	1.10
*38	F	37		0.84	25.0	3.45	8.81	1.44	1.22	1.17
			44	2.58	39.0	5.38	17.50	0.73	0.79	0.92
**12	M	62		1.02	37.2	5.14	13.00	1.71	1.55	1.10
			31	3.34	81.0	11.18	33.07	1.14	1.21	0.94
			64	4.54	85.2	11.76	38.61	0.88	1.04	0.85
**15	M	44		1.33	40.7	5.62	15.36	1.44	1.41	1.02
			24	2.43	81.5	11.25	30.77	1.57	1.54	1.02
			37	4.39	98.7	13.62	43.08	1.06	1.20	0.88
**37	M	49		---	---	---	---	---	---	---
			54	5.01	111.5	15.39	48.74	1.04	1.19	0.88
**39	M	67		---	---	---	---	---	---	---
			370	2.27	56.0	7.73	21.33	1.16	1.15	1.01

*Nuclear extract

**Minot Murphy diet

Treated Pernicious Anemia—Table III presents results of studies on 8 cases of pernicious anemia before and after receiving specific treatment. The figures in italics are the results before treatment. The first four cases (11, 33, 34 and 38) were treated with the nuclear extract of Jones, Phillips, Larzell, and Nokes^{50, 51}. The last four (12, 15, 37 and 39) were treated with the liver diet of Minot and Murphy.¹ Note that the color and volume indexes return to normal after a few weeks on either treatment. No changes as marked as these were observed in spontaneous remissions of untreated cases. Knowing this it is necessary to find out whether the patient has been taking such therapy before relying on a normal or low color and volume index to exclude the diagnosis of pernicious anemia.

Anemia of Chronic Blood Loss—Table IV presents results of the study of ten cases in which chronic hemorrhage was the only discoverable factor tending to produce anemia. Note that there is a marked tendency for all of the indexes to be low. As will be shown later this is the only type of anemia, with the exception of the chlorotic type, in which the saturation index is low. The highest saturation index is 0.91 (Case 45) and this may well have been due to the large blood transfusion nine days before.

In Table V are presented fifteen studies on thirteen cases in which chronic hemorrhage was the chief, although probably not the only, factor tending to produce anemia. The results are similar to those in Table IV. In only two cases is the saturation index well within normal limits and in one of these (Case 60) the duration of the bleeding was unknown, while in the other (Case 61) the duration was only two months and a second determination a week or so later, just after a hysterectomy had been performed, showed a decreasing index. *This low saturation index occurs so rarely in any other type of anemia that, in our opinion, chronic hemorrhage should be considered as the most probable cause of any anemia showing a saturation index below 0.85, until it has been definitely excluded.* This is a very important diagnostic point, for it is thus possible to reach a correct conclusion the first day the patient is seen, even though the bleeding may have stopped previously or though several days' observation may be necessary to detect its source. A high saturation index, on the other hand, is a point against an anemia being due largely to chronic loss of blood even though bleeding is occurring. The diagnostic value of this index was apparently overlooked prior to the publication of our preliminary report,⁵ although it is clearly illustrated in Capps's article²⁵ (see his Table XIV) and also in the results of other writers on this subject. More recently, Wintrobe⁴⁶ has confirmed this observation. A low icterus index was characteristic of this group of cases.

Anemias Due to Infection—It has been customary to group these under the heading of hemolytic anemias, but it appears to the authors that this is unjustifiable, since depression of bone marrow activity by circulating toxin and sensitization of red cells to normal blood destroying mechanisms probably play a part in the production of these anemias, also. The relative proportion of the anemia due to these different factors undoubtedly varies with different sites and types of infection and with varying virulence of the organism.

TABLE IV
CHRONIC HEMORRHAGE ONLY

CASE NO	SEX	AGE	RED CELL COUNT, MILLIONS PER C MM	HEMOGLOBIN PER GRAMS PER CENT	VOLUME OF CELLS PER 100 C C	COLOR INDEX	VOLUME INDEX	SATURATION INDEX	DIAGNOSIS AND REMARKS
*40	M	63	1.45	18.1	2.49	8.61	0.58	0.72	Papillodenoma of bladder
41	M	59	2.02	25.5	3.52	11.14	0.59	0.67	Hemorrhoids, 15 yr duration
42	F	57	2.45	27.0	3.73	14.93	0.53	0.71	Hemorrhoids, 30 yr duration
43	M	44	4.32	50.0	6.90	25.25	0.54	0.71	Hemorrhoids
44	M	60	2.31	32.3	4.47	15.27	0.66	0.81	Extreme hematuria
45	F	32	2.64	47.3	6.53	21.20	0.86	0.95	Metrorrhagia secondary to uterine fibroids**
46	F	30	4.21	58.5	8.07	28.47	0.67	0.79	Essential menorrhagia, 1 yr duration
47	F	24	4.73	58.5	8.07	28.92	0.60	0.71	Essential menorrhagia
48	F	15	3.78	60.4	8.34	29.41	0.77	0.90	Essential menorrhagia, 1 yr duration***
49	M	40	3.53	57.8	7.98	30.66	0.77	1.06	Gastric ulcer, history of tarry stools for many months
Minimum			1.45	18.1	2.49	8.61	0.53	0.67	
Maximum			4.73	60.4	8.34	30.66	0.86	1.06	
Average			3.14	43.5	6.01	21.42	0.66	0.80	

*Source undetermined

Recovery followed spontaneous cessation

Diagnosis confirmed by necropsy

**Large transfusion nine days before

***Bleeding ceased eleven days before count

TABLE V
CHRONIC HEMORRHAGE FOR CHIEF FACTOR

CASE NO.	SEX	AGE	RED BLOOD COUNT, MILLIONS PER CCM	HGB. GRAMS PER 100 CC	VOLUME OF OPTIC DISC, 100 CC	COLOR INDEX	VOLUME INDEX	SATURATION INDEX	DIAGNOSIS AND REMARKS
*50	M	69	1.47	3.18	10.85	0.71	0.90	0.82	Carcinoma of stomach
*51	M	59	3.20	3.93	16.16	0.12	0.63	0.67	Carcinoma of stomach
*52	M	60	1.96	4.15	13.78	0.72	0.86	0.81	Carcinoma of stomach
*53	M	72	2.56	4.27	16.63	0.37	0.79	0.72	Carcinoma of stomach
*54	M	13	2.70	4.68	16.81	0.59	0.76	0.78	Carcinoma of stomach
*55	M	58	3.26	5.11	16.67	0.54	0.62	0.86	Carcinoma of stomach
			3.78	5.76	21.78	0.52	0.70	0.74	
*56	M	52	2.61	5.19	20.15	0.71	0.91	0.75	Carcinoma of stomach
57	M	51	3.08	4.97	18.73	0.55	0.71	0.74	Carcinoma of pancreas, melena
58	M	51	2.28	5.56	19.61	0.83	1.05	0.79	Peptic ulcer, chronic infectious arthritis
59	F	55	3.50	6.28	21.21	0.63	0.80	0.78	Bright red and dark blood in stools for six years, cause undetermined Arthritis, too young, cause undetermined
60	F	67	3.31	6.80	20.62	0.71	0.72	0.99	Carcinoma of rectum, duration of bleeding unknown
61	F	50	3.18	8.73	21.62	0.87	0.82	1.05	Metrorrhagia secondary to fibroid uterus**
			3.38	7.70	26.27	0.77	0.92	0.86	Abscessed teeth
62	M	45	3.66	9.83	33.08	0.91	1.10	0.83	Chronic ulcerative colitis, stools streaked with blood
Minimum			1.47	3.18	10.85	0.12	0.62	0.67	
Maximum			3.78	9.83	33.08	0.91	1.10	1.05	
Average			2.95	5.76	20.01	0.67	0.82	0.81	
Average of Tables IV and V			3.01	5.86	20.57	0.67	0.92	0.92	

*Diagnosis confirmed by necropsy

**Hysterecctomy before counts

TABLE VI
INFECTION ONLY

CASE NO	SEX	AGE	RED CELL COUNT, MILLIONS PER CMM	HEMOGLOBIN PER GRAMS PER 100 C C	VOLUME OF CELLS PER 100 C C	COLOR INDEX	VOLUME INDEX	SATURATION INDEX	DIAGNOSIS AND REMARKS
63	M	16	0.81	20.5	2.83	1.19	1.08	1.10	Chronic diffuse nephritis
64	M	57	2.30	55.3	7.63	1.13	1.09	1.04	Chronic diffuse nephritis
65	F	16	3.76	61.5	8.49	0.79	0.76	1.03	Chronic diffuse nephritis
66	F	25	2.62	63.7	8.79	1.17	1.10	1.07	Chronic diffuse nephritis
67	F	7	3.89	78.9	10.88	0.98	0.95	1.03	Early chronic diffuse nephritis
68	M	29	2.84	52.3	7.22	0.86	0.98	0.88	Subacute glomerular nephritis, subacute septic arthritis of knee
*69	M	48	3.23	67.0	9.25	0.97	0.89	1.10	Subacute glomerular nephritis, in uremia
*70	M	54	1.74	44.0	6.07	1.18	1.23	0.96	Chronic cholecystitis and chronic fibrous peritonitis
71	F	39	4.24	48.5	6.69	0.53	0.59	0.93	Chronic cholecystitis with stones. Ellipt cells
72	M	24	4.10	44.8	6.18	0.53	0.58	0.90	Subacute bacterial endocarditis with focal embolic nephritis
*73	F	26	3.86	54.1	7.46	0.66	0.74	0.88	Subacute bacterial endocarditis, chronic diffuse nephritis
			2.60	59.0	8.14	1.07	0.90	1.18	
74	M	52	2.69	60.2	8.31	1.05	1.24	0.85	Subacute bacterial endocarditis, hemolytic strep found on blood culture

*Diagnosis confirmed by necropsy

TABLE VI—Continued

CASE NO.	SEX	AGE	RED CELL COUNT, MILLIONS PER C.C.	HEMOGLOBIN, GRAMS PER 100 C.C.	VOLUME OF CELLS, PER 100 C.C.	COLOR INDEX	VOLUME INDEX	SATURATION INDEX	DIAGNOSIS AND REMARKS
75	M	31	1.76	36.0	11.87	36.76	0.89	0.98	Subacute bacterial endocarditis, Strept viridans found in blood
76	F	50	3.52	65.5	9.04	30.09	0.90	0.99	Endocarditis and bacteremia due to Staph hemolyticus
77	M	76	2.49	55.0	7.59	23.25	1.01	1.14	Pyonephrosis with mening secondary to prostatic obstruction
78	F	37	1.71	61.5	8.19	29.15	0.63	0.72	Chronic sinusitis, history suggestive of chole cystitis, Gramm Cole neg
79	F	28	1.72	62.3	8.60	31.12	0.64	0.77	Ellipt cells
80	M	77	1.50	67.1	9.26	29.61	0.72	0.77	Chronic sinusitis, ellipt cells
81	M	26	1.31	67.1	9.30	30.18	1.03	1.19	Chronic sinusitis with "sinus" bronchitis
82	F	18	3.18	76.6	10.57	30.02	0.75	0.83	Bilat acute conductive tuberculosis, no cavity, ellipt cells
83	M	68	1.17	87.2	12.03	36.62	1.06	1.00	Acute rheumatic fever with active mitral endocarditis
84	M	33	3.90	88.1	12.15	26.75	0.98	1.07	Lobar pneumonia, nine days after crisis
85	M	11	1.76	89.0	12.28	12.17	1.06	1.18	Lucetic (miscellaneous) hepatitis with jaundice
Minimum			0.81	20.50	2.83	7.18	0.83	0.58	Subacute hepatitis, catarrhal jaundice
Maximum			1.76	89.00	12.28	12.17	1.19	1.21	
Average			1.48	62.90	8.68	26.37	0.90	0.95	

In Table VI are presented twenty-six studies of twenty-three cases in which the anemia was due, as far as could be determined, solely to infection. They are arranged by disease groups. The first seven are cases of subacute glomerular or chronic diffuse nephritis. Our studies confirm the statements of Brown and Roth,^{52, 53} namely, that anemia is almost constantly associated with this type of nephritis, that its severity runs parallel with the severity of the nephritis, and that the nature of the anemia (normal indexes, low reticulocyte count and icterus index, absence of urobilinogen in the urine) suggests as the chief etiologic factor bone marrow depression rather than hemolysis. Our results disagree with those reported by Ashe,⁵⁴ probably because he reports results obtained with the Dare hemoglobinometer which has been repeatedly shown to have limits of error exceeding plus or minus 35 per cent and thus is worse than valueless for color and saturation index calculations.

The normal saturation indexes confirm the experimental observations that the loss of blood cells in the urine is far too slight (always less than 5 c c, usually less than 1 c c, of blood per day) to be a factor in the production of the anemia. This anemia in chronic diffuse nephritis is so constant that a hematologic study is of great value in the differential diagnosis from hypertensive cardiovascular renal disease in which anemia rarely occurs.

The other conditions listed in this table require no special comment except to point out that these diagnoses should always be considered when searching for the cause of an anemia associated with normal or low color and volume indexes and with a normal saturation index.

Note that while the color and volume indexes may be either normal or low in this group, they never exceed the upper normal limits and that the saturation index is consistently normal, only two estimations below 0.85 being recorded.

Table VII shows results on eight cases in which infection was the chief factor in the production of the anemia. The results are similar to those in the previous table, but the saturation indexes average lower, due to the fact that hemorrhage was a complication in four cases.

Note the frequency with which elliptical erythrocytes were observed in these cases. Case 79 is the same as Adriana N. reported by Hunter and Adams.⁵⁵ They demonstrated similar elliptical cells in members of three generations of this same family, some of whom had no anemia. Lawrence⁵⁶ had previously reported the finding of elliptical and sickle-shaped erythrocytes in the blood of white persons. A search through our slides revealed typical elongated cells in certain cases of almost every type of anemia, hence we were forced to agree with Huck⁵⁷ that this is either merely one type of poikilocytosis or, which is more probable, that it is a familial anomaly of red cell form which is exaggerated by coexistent anemia but is not a factor in the production of that anemia. It is readily differentiated from true sickle-cell anemia which occurs only, as far as is known, in the negro race.

Anemia Associated With Diseases of the Blood-Forming Organs—Table VIII presents results of fourteen studies on twelve cases. Note that, with few exceptions, the indexes are normal. In the one case (98) in which the color index was high, the white and differential cell count at once gave the

TABLE VII
INFECTION AND CHIEF FACTOR

CASE NO	SEX	AGE	RED CELL COUNT, MILLIONS PER CMM	HGB PER CENT	HGB GRAMS PER 100 G	VOLUME OF CELLS PER 100 G	COLOR INDEX	VOLUME INDEX	SATURATION INDEX	DIAGNOSIS AND REMARKS
86	F	22	2.61	31.5	1.76	15.75	0.61	0.70	0.91	Frontal sinusitis Occasional epistaxis Erythroid cells
87	F	10	3.05	19.3	6.80	21.15	0.78	0.81	0.97	Sinusitis, cholecystitis, peptic ulcer with early carcinoma Erythroid cells
88	F	21	3.97	17.3	6.53	22.66	0.57	0.66	0.87	Puerperal sepsis plus postpartum hemorrhage Erythroid cells
89	F	16	1.22	51.0	7.15	25.71	0.62	0.71	0.87	Pharyngitis and bronchopneumonia Pregnant at term Erythroid cells
90	F	28	3.51	19.5	6.83	21.17	0.67	0.79	0.85	Chronic infectious arthritis Amebic dysentery (Diagnosed one year later)
91	M	41	3.66	67.7	9.35	30.77	0.87	1.02	0.95	S viridans bacteremia Unexplained melena
92	M	38	3.50	75.1	10.40	33.33	1.01	1.16	0.87	S viridans bacteremia, abscess of spleen, and subacute bacterial endocarditis Bleeding hemorrhoid
93	F	15	1.02	68.5	9.15	31.76	0.82	0.92	0.89	Tertiary syphilis Ovarian cyst
Minimum			2.61	11.5	1.76	15.75	0.57	0.66	0.95	
Maximum			1.22	76.1	10.40	33.33	1.01	1.16	0.97	
Average			3.57	55.8	7.69	25.67	0.75	0.85	0.88	
Average of Tables VI and VII			3.50	61.2	8.69	26.20	0.86	0.92	0.91	

*Diagnosis confirmed by necropsy

TABLE VIII
DISEASES OF THE BLOOD FORMING ORGANS

CASE NO	SEX	AGE	RED CELL COUNT, MILLIONS PER C.M.M.	HEMOGLOBIN PER C.C.	VOLUME OF CELLS PER 100 C.C.	COLOR INDEX	VOLUME INDEX	SATURATION INDEX	DIAGNOSIS AND REMARKS
94	M	67	0.93	24.0	3.31	1.21	1.22	0.99	Chronic lymphoid leucemia
95	F	49	1.90	40.8	5.63	1.01	1.17	0.86	Chronic lymphoid leucemia
96	F	68	4.02	80.0	11.04	0.96	1.08	0.89	Chronic lymphoid leucemia
97	M	57	3.38	92.3	12.74	1.21	1.16	1.05	Chronic lymphoid leucemia
98	M	6	1.12	32.0	4.42	1.34	1.23	1.09	Acute myeloid leucemia
*99	M	60	2.41	37.0	5.11	0.72	0.92	0.78	Acute myeloid leucemia
100	M	26	2.52	48.3	6.73	0.91	1.01	0.90	Chronic myeloid leucemia
*101	F	44	4.34	91.2	12.59	0.97	0.88	1.10	Chronic myeloid leucemia
*102	F	13	3.50	85.5	11.80	1.18	1.18	1.00	Banti's disease
			2.51	57.0	7.87	1.10	1.12	0.98	
*103	F	34	3.04	60.1	8.34	0.96	1.01	0.95	Banti's disease
*104	F	36	3.90	72.7	10.04	0.90	0.89	1.01	Hodgkin's disease
*105	M	57	4.38	71.5	9.87	0.79	0.86	0.92	Hodgkin's disease
			3.27	81.3	11.22	1.17	1.08	1.08	
Minimum			0.93	24.0	3.31	0.72	0.86	0.78	
Maximum			4.38	92.3	12.74	1.34	1.23	1.10	
Average			2.97	62.5	8.62	1.03	1.06	0.97	

*Diagnosis confirmed by necropsy

**Confirmed by pathological examination of spleen removed by operation

differential diagnosis from pernicious anemia. There is some doubt whether these indexes are correct as adult standards were used for calculation since no satisfactory standards exist for children of this age (six years). In the one case (99) in which the saturation index was low, hemorrhage could not be excluded.

Miscellaneous Anemias—Table IX contains twenty-eight studies on a very interesting group of nineteen cases of anemia of several types, the number of each type being too small to warrant a separate table.

The first two cases are the only chlorosis cases that we were able to find in a period of seven years. These were mild cases and the findings resemble those in the chronic hemorrhage group. In more severe cases Capps,²⁶ Bonninger²¹ and others have found still lower color, volume and saturation indexes.

Malignant tumors (Cases 108 to 113) showed practically normal saturation indexes in contrast with the low indexes of carcinoma cases with hemorrhage (Table V). Case 111 deserves special mention as it was the only case with high indexes (aside from one leukemia) in which the diagnosis of pernicious anemia seemed doubtful. This patient was first seen by Dr N. W. Jones and the diagnosis of multiple myeloma (proved correct at necropsy) was made by him chiefly on the basis of the typical roentgen-ray findings in the bones. His laboratory also found a high color and volume index. There was nothing in the clinical picture either particularly to suggest or to rule out pernicious anemia. Necropsy revealed in addition to the multiple myeloma, a marked megaloblastic hyperplasia of the bone marrow but the spleen was not enlarged and showed none of the changes common in pernicious anemia. We are undecided as to whether this is a coincidence of multiple myeloma and pernicious anemia, which seems very improbable or whether the blood picture of pernicious anemia was produced by the involvement of the bone marrow. The latter would seem more probable were it not for the fact that the other malignant tumors in which the bone marrow was involved showed no elevation of the color and volume indexes. We have been unable to find reports of other cases of multiple myeloma in which sufficiently accurate hematologic studies were made to form a basis for conclusions. That anemia is associated as a rule with multiple myeloma has been shown²⁵ repeatedly.

The mode of production of anemia associated with malignant tumors is unquestionably varied and complex. Hemorrhage, bone marrow metastases, toxin production (?) and secondary infection may all play a part and the effects of toxin production and secondary infection may be local blood destruction, generalized hemolysis, or depression of bone marrow function. In our experience, hemorrhage and bone marrow metastases are by far the most important since anemia occurs late or not at all in those tumors in which these can be definitely excluded.

The two cases (114 and 115) of malaria show normal indexes. This agrees with the observation of Wintrobe.¹⁴ Here the chief factor in the production of the anemia is unquestionably the destruction of red blood cells within the blood stream. Similar findings were observed in one case (116) of lead poisoning and one case (117) of acetanilid poisoning. The case of lead poisoning is noteworthy in that as many as eighteen nucleated red cells,

TABLE IX
MISCELLANEOUS ANEMIAS

CASE NO	SEX	AGE	RED CELL COUNT, MILLIONS PER C MM	HEMOGLOBIN PER CENT	GRAMS PER 100 C C	VOLUME OF CELLS PER 100 C C	COLOR INDEX	VOLUME INDEX	SATURATION INDEX	DIAGNOSIS AND REMARKS
106	F	18	4.63	65.2	9.00	31.41	0.68	0.79	0.86	Chlorosis
107	F	21	4.96	69.3	9.56	33.33	0.67	0.78	0.86	Chlorosis
*108	M	65	2.33	38.1	5.25	17.73	0.77	0.93	0.82	Primary carcinoma of prostate with bone metastases, paraplegia, and sanguinopurulent cystopyonephrosis
*109	M	64	2.58	43.8	6.04	18.85	0.80	0.89	0.89	Annular carcinoma of the rectum with bilateral hydronephrosis No blood in stools
*110	M	66	2.49	59.7	8.24	24.57	1.13	1.21	0.94	Malignant tumor of lymph glands metastasizing to bone
*111	M	71	2.72	72.4	10.00	32.34	1.25	1.45	0.86	Multiple myeloma (Pernicious anemia?)
112	F	71	4.75	78.0	10.76	32.26	0.79	0.79	1.00	Carcinomatosis Primary site undetermined
113	M	63	4.32	82.0	11.32	34.43	0.89	0.97	0.92	No blood loss Scurious carcinoma of the stomach No loss of blood
114	M	32	3.46	67.9	9.37	29.68	0.92	1.05	0.88	Inoculation malaria for general piresis
115	M	18	3.38	73.5	10.14	30.41	1.02	1.10	0.93	Twenty days after inoculation
116	M	32	2.96	59.0	9.14	22.61	0.91	0.93	1.00	Acquired tertian malaria Acute exacerbation of a chronic lead poisoning

*Diagnosis confirmed by necropsy

TABLE IV—Cont'd

CASE NO.	SEX	AGE	RED CELL COUNT, MILLIONS PER C.C.	HEMOGLOBIN, GMS. PER 100 C.C.	VOLUME OF BLOOD, C.C.	COLOR INDEX	VOLUME INDEX	SATURATION INDEX	DIAGNOSIS AND REMARKS
117	M	47	2.61	7.66	21.15	1.00	1.14	0.87	* Acetaminid poisoning Polyglandular deficiency
*119	F	15	3.67	10.93	14.74	1.01	1.15	0.88	
			3.19	9.70	30.65	1.06	1.12	0.95	
			3.14	9.77	30.77	1.04	1.14	0.92	Painous anemia of pregnancy
			3.75	11.69	37.04	1.09	1.15	0.95	
			0.92	3.14	8.98	1.19	1.14	1.05	
119	F	33	1.60	6.19	19.20	1.42	1.10	1.02	Sickle cell anemia
120	F	7	2.89	51.5	17.02	1.19	0.95	1.26	Acute hemorrhage from duodenal ulcer
*121	M	67	1.71	5.71	16.75	1.13	1.18	0.95	enema of head of pancreas
122	M	47	2.75	8.17	23.82	1.01	1.01	1.00	Acute large hemorrhage from the bowel
123	F	19	2.83	9.44	25.00	1.13	1.03	1.10	Acute hemorrhage from the bowel
124	M	25	5.26	112.1	43.10	1.00	1.00	1.00	Acute hemorrhage from the bowel
			1.76	100.6	38.51	1.04	1.03	1.01	Acute hemorrhage from the bowel
			1.70	95.7	38.15	0.96	0.99	0.97	Acute hemorrhage from the bowel
			1.75	99.1	39.24	0.97	1.00	0.97	Acute hemorrhage from the bowel
			1.99	102.0	39.49	0.96	0.97	0.99	Acute hemorrhage from the bowel
			1.92	111.8	42.89	1.07	1.06	1.00	Acute hemorrhage from the bowel

many of them typical megaloblasts, were seen in counting 100 white cells. This case would unquestionably have been diagnosed as pernicious anemia from a study of the stained smear had the criteria given in most texts been used.

Case 118* had a very interesting polyglandular deficiency in which hypofunction of the thyroid and suprarenal glands predominated. The counts extend over a period from February, 1925, to November, 1926. Note the very severe terminal anemia for which even at postmortem examination no cause other than the glandular deficiency could be found. Notwithstanding the statements, frequently made, that the anemia of myxedema may simulate that of pernicious anemia, her color and volume indexes remained normal throughout. This agrees with the observations of MacKenzie.⁶⁰

Case 119 is our only case of pernicious anemia of pregnancy. Note the high color and volume indexes.

Case 120 is probably the first case of sickle-cell anemia in which color, volume and saturation indexes have been calculated. However, we had to use adult white standards, since those for negro children are not known. It seems probable that all the indexes will be normal in sickle-cell anemia if calculated from correct standards.

In the four cases (121 to 124) of acute hemorrhage all the indexes were normal. Case 124 is our only case of acute blood loss in a healthy young man, and is particularly interesting in that records are available before and at intervals after donating 875 cc of blood. This loss did not reduce the red cell count or the hemoglobin below the lower limits of normal (no anemia). All blood findings returned to practically their previous level within a month.

Nonanemic Group—There were a large number of cases originally diagnosed as anemia which could not be used in this paper as true anemia cases, from these we have selected 20 as being particularly instructive (see Table X).

We would define anemia as a decrease in either the red cells or hemoglobin or both below the lower normal limits for persons of the patient's sex and age group. It is possible that in some of these cases there is a relative decrease as compared to the normal level for that particular person (cf., Case 124) but as previous determinations on these individuals are lacking, it seems only fair to segregate them. This is a precaution which other writers have not observed.

It is noteworthy that the first cases (125 to 130, inclusive) had been erroneously diagnosed as having pernicious anemia. They will be discussed in detail in connection with Table XII.

The next two patients (131 and 132) had been told within a day or so of the date of our study that they had anemia which required treatment. In each instance, the basis for the diagnosis of anemia was a Dacie hemoglobin estimation of 65 per cent.

Cases 133 and 134 are examples of the condition called by the Germans "Scheinanämie" or "apparent anemia." They had no symptoms but looked so strikingly pale that it was difficult to believe that anemia was absent.

Cases 135 to 141 are examples of conditions in which anemia sometimes occurs, but which, in these instances, show no anemia. In our experience

*This case is to be reported by Dr. Rush.⁵⁰

TABLE X
No ANEMIA

CASE NO	SEX	AGE	RED CELL COUNT, MILLIONS PER CUBIC MM	HGB, GRAMS PER 100 CC	VOLUME OF GLASS, CC	COLOR INDEX	VOLUME INDEX	SATURATION INDEX	DIAGNOSIS AND REMARKS
*125	M	17	5.51	15.26	18.16	0.91	1.06	0.88	Combined system disease
*126	M	11	1.70	14.15	12.13	1.01	1.10	0.91	Combined system disease
*127	F	6 1/2	1.10	11.29	36.02	0.92	0.95	0.96	Hyperplastic exophthalmic goiter
*128	M	5 1/2	5.06	15.39	12.07	1.03	1.01	1.02	Hyperplastic cardiovascular renal disease
*129	M	12	5.98	18.55	16.55	1.06	0.95	1.11	Venital tumor Appeared anemic later for three months
**130	F	13	5.59	15.76	17.01	0.99	0.98	1.01	Chronic cholecystitis (Thought to be pernicious anemia and ilect)
131	M	11	1.95	11.77	11.36	1.01	1.09	0.93	Normal Supposed to be anemic
132	F	18	1.19	13.18	10.65	1.03	1.05	0.98	Normal Supposed to be anemic
133	F	10	1.61	11.50	11.12	1.02	1.08	0.91	Normal Appeared anemic
134	F	21	1.97	11.59	17.97	0.82	0.80	0.92	Normal Appeared anemic
135	F	16	1.61	11.15	19.95	1.07	1.00	1.07	Same, three years later
136	M	70	1.12	11.01	15.26	1.18	1.00	1.18	Tapeworm Taenia saginata
**137	M	29	1.71	13.30	18.23	0.95	0.98	0.97	Hypertensive cardiovascular renal disease
138	M	23	5.19	17.17	19.17	1.08	1.10	0.99	Gastroenteritis, hepatitis, and icterus from poisoning with bootleg liquor
139	F	19	1.59	12.15	31.15	0.90	0.91	0.99	Typhoid fever—two weeks
**140	M	21	1.52	11.59	36.30	0.90	0.93	0.96	Infectious mononucleosis
141	F	26	5.71	13.22	10.11	0.79	0.86	0.92	Pick's disease Active tuberculous pericarditis
142	F	20	5.72	12.18	10.51	0.75	0.82	0.90	Rheumatic mitral stenosis and regurgitation (Thought to be bacterial endocarditis)
143	M	12	5.12	11.65	16.31	0.80	0.93	0.96	Normal with slight chlorotic tendency Sister of No 10
144	F	17	1.96	11.73	10.87	1.01	1.00	1.00	Normal Son of No 71
			5.01	11.28	10.07	1.00	0.93	1.07	Normal Daughter of No 71

*Previous diagnosis of pernicious anemia
**That node confirmed by necropsy

TABLE XI
PERNICIOUS ANEMIAS CLINICALLY MISDIAGNOSED

CASE NO	SEX	AGE	RED CELL COUNT, MILLIONS PER C MM	HEMOGLOBIN PER GRAMS PER 100 C C		VOLUME OF CELLS PER 100 C C	COLOR INDEX	VOLUME SATURATION INDEX		ORIGINAL DIAGNOSIS
				PER CENT	100 C C			INDEX	INDEX	
*3	M	24	0.53	16.8	2.32	6.58	1.50	1.53	0.98	Lung abscess with anemia
10	F	16	1.02	34.7	4.79	14.33	1.64	1.63	1.01	Familial hemolytic icterus
15	M	44	1.33	40.7	5.62	15.36	1.44	1.41	1.02	Carcinoma of stomach
21	M	78	1.51	46.1	6.41	17.25	1.44	1.39	1.04	Anemia of unknown type
*23	F	50	1.77	50.7	7.00	19.58	1.38	1.29	1.07	Anemia (not pernicious)
29	M	56	2.42	64.9	8.95	26.84	1.24	1.36	0.91	Chronic cholecystitis with anemia
31	F	66	2.76	72.0	9.94	32.50	1.26	1.37	0.92	Chronic cholecystitis with anemia
32	M	45	2.53	74.0	10.21	32.98	1.37	1.59	0.86	Chronic cholecystitis with anemia

*Diagnosis confirmed by necropsy

anemia is the exception in hypertensive cardiovascular renal disease, infectious mononucleosis and in tuberculosis which is uncomplicated by hemorrhage or secondary infection. The absence of anemia in infectious mononucleosis is an important point in differentiating it from acute lymphatic leucemia which it may closely resemble in other respects.

Cases 143 and 144 were examined solely because the mother (Case 71) showed numerous elliptical red cells, but their bloods contained only a few such cells.

Pernicious Anemias Clinically Misdiagnosed—More than these eight cases (Table XI) might have been included had we not strictly adhered to our rule to discard all those cases in which a reasonably certain diagnosis was not ultimately reached by other methods than a study of the color volume and saturation index. The color and volume indexes were high in every case at the time when the erroneous diagnosis (see last column of the table) was made. If the criterion of a color or volume index above 1.25 had been used by the clinician there would not have been this failure of correct diagnosis in 20 per cent of the pernicious anemia cases.

Erroneous Clinical Diagnosis of Pernicious Anemia—These 13 cases are summarized in Table XII. Note that if reliance had been placed on the color and volume index, question could have arisen only in Case 70 and in that case the indexes do not exceed the highest observed in perfectly healthy persons. Nevertheless, each of these cases was diagnosed pernicious anemia at the time the first recorded studies were made. The correct diagnoses ultimately reached are noted in the last column. Observe that the conditions which are likely to be confused clinically with pernicious anemia are with the exception of carcinoma of the stomach, not those ordinarily mentioned in texts as difficult to differentiate.

Case 92 was demonstrated twice to senior medical students by well-trained internists as a typical case of pernicious anemia. Following the detection of normal color and volume indexes, positive blood cultures for *Streptococcus viridans* were secured and a postmortem examination revealed a typical subacute bacterial endocarditis with the rare complication of an abscess in the spleen from which *Streptococcus viridans* was cultured.

This initial clinical diagnostic error of 13 cases out of a possible 106, or 12 per cent, is to be contrasted with an error of only two cases (98 and 111) or 2 per cent, if both a color and volume index below 1.25 be taken as the criterion for excluding the diagnosis of pernicious anemia. Here again this series might have been increased by less strict adherence to our rule for discarding cases.

DISCUSSION

It is regretted that opportunity did not present for the study of the anemias of sprue, *Dibothriocephalus latus* infestation, aplasia of the bone marrow or familial hemolytic icterus. It seems however, definitely established that the color and volume indexes may be high in some cases of anemia associated with sprue⁴¹ or infestation with *Dibothriocephalus latus*.⁴² It has not however been sufficiently emphasized that the pernicious anemia syndrome is the exception rather than the rule in patients harboring the broad tapeworm.⁴²

TABLE XII
ERRONEOUS CLINICAL DIAGNOSIS OF PERNICIOUS ANEMIA

CASE NO	SEX	AGE	RED CELL COUNT, MILLIONS PER C.M.M.	HEMOGLOBIN PER GRAMS PER 100 C.C.	VOLUME OF CELLS PER 100 C.C.	COLOR INDEX	VOLUME INDEX	SATURATION INDEX	CONCURRENT DISEASES	
*92	M	38	3.50	75.4	10.40	33.43	1.01	1.16	0.87	Subacute bacterial endocarditis with Streptococcus viridans abscess in spleen and big toe; Bleeding hemorrhoids
42	F	57	2.45	27.0	3.73	14.93	0.53	0.71	0.75	Bleeding hemorrhoids
*52	M	60	1.96	30.1	4.15	13.78	0.72	0.86	0.54	Carcinoma of stomach
54	M	43	2.70	33.9	4.68	16.84	0.59	0.76	0.78	Carcinoma of stomach
*55	M	58	3.26	37.2	5.14	16.67	0.54	0.62	0.86	Carcinoma of stomach
			3.78	41.7	5.76	21.78	0.52	0.70	0.74	
*70	M	54	1.74	44.0	6.07	17.59	1.18	1.23	0.96	Chronic cholecystitis and chronic fibrosis peritonitis
71	F	39	4.24	48.5	6.69	21.54	0.55	0.59	0.93	Chronic cholecystitis with stones
			4.10	44.8	6.18	20.59	0.53	0.58	0.90	
*138	F	33	5.59	114.2	17.76	47.04	0.99	0.98	1.01	Chronic cholecystitis (No anemia)
125	M	47	5.54	110.6	15.26	48.16	0.94	1.06	0.88	Combined system disease (No anemia)
126	M	44	4.70	104.0	14.35	42.43	1.04	1.10	0.94	Combined system disease (No anemia)
127	F	63	4.40	81.8	11.29	36.02	0.92	0.95	0.96	Toxic hyperplastic (exophthalmic) goiter (No anemia)
128	M	54	5.06	111.5	15.39	42.07	1.03	1.01	1.02	Hypertensive cardiovascular renal disease (No anemia)
129	M	42	5.98	134.4	18.55	46.55	1.06	0.95	1.11	Ventricular hernia (No anemia)

*Diagnosis confirmed by necropsy

*Diagnosis confirmed by necropsy

It seems certain that the color volume and saturation indexes are normal in true aplastic anemia, although we have studied only one case (not included in the series). If Alder's⁵² observation that the cell volume (volume index) is normal while the cell diameter is decreased in familial hemolytic icterus, can be substantiated, this will be one disease in which the tedious determination of the average red cell diameter adds to the information secured by the easily determined volume index.

Murphy and Fitzhugh⁶⁴ have published data on a considerable number of anemias from which the color volume and saturation indexes may be calculated. Their results agree quite well with those herein reported. The tendency to a low saturation index in chronic hemorrhage is strikingly demonstrated by calculation of these indexes from the data in their table although they do not comment on that fact. The few exceptions are probably due to the occasional use of the Dare or Tallqvist hemoglobin methods, although most of their data were secured by the use of an accurate method. We heartily agree with their conclusion that the determination of cell volume is a more simple means of determining the average size of the red cell than is the measurement of the mean cell diameter.

We regret that the time and funds available for this study did not seem to warrant a determination of the total blood and plasma volumes in each case, as this would have undoubtedly contributed further information of value.

Criteria for the differential diagnosis of 28 types of anemia based chiefly on the other laboratory and clinical studies in this series of cases are summarized in a table in another article,⁶³ together with a discussion of the fundamental causes of anemia.

SUMMARY

The literature on the color, volume and saturation indexes is reviewed. Detailed results, obtained by methods of research accuracy, are reported for hemoglobin, red cell count, cell volume, color index, volume index and saturation index on 144 cases of anemia and related conditions.

CONCLUSIONS

1 Capps's work on the color and volume index in anemias has never received the credit it deserves.

2 Bonninger (1919) was the first to calculate in essentially its present form the ratio which is now called the saturation index, although Haden (1923) deserves credit for coining this term and for reawakening interest in these indexes in English-speaking countries.

3 The most characteristic change in pernicious anemia is the preponderance of macrocytes in the blood. This is most easily recognized by the volume index determination.

4 The high color index in pernicious anemia is due to the increase in size of the cell, not to increased concentration of hemoglobin within the cell. In other words, the saturation index is normal, and true hyperchromia does not occur either in pernicious anemia or in any other condition so far studied.

TABLE XII
ERRONEOUS CLINICAL DIAGNOSIS OF PERNICIOUS ANEMIA

CASE NO	SEX	AGE	RED CELL COUNT, MILLIONS PER C MM	HEMOGLOBIN PER CENT	VOLUME OF CELLS PER 100 C C	COLOR INDEX	VOLUME INDEX	SATURATION INDEX	CORRELATIVE DIAGNOSIS
*92	M	38	3.50	75.1	33.33	1.01	1.16	0.87	Sub-acute bacterial endocarditis with Streptococcus viridans abscess in spleen and biliary tree; Bleeding hemorrhoids
42	F	57	2.45	27.0	14.93	0.53	0.71	0.75	Bleeding hemorrhoids
*52	M	60	1.96	30.1	13.78	0.72	0.86	0.54	Carcinoma of stomach
54	M	43	2.70	33.9	16.84	0.59	0.76	0.78	Carcinoma of stomach
*55	M	58	3.26	37.2	16.67	0.54	0.62	0.86	Carcinoma of stomach
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					46.55	1.06	0.95	1.11	

*Diagnosis confirmed by necropsy

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5 If either the color or volume index is above 1.25, the patient, with rare exceptions, will prove to have pernicious anemia

6 Such a high color and volume index constitutes a definite indication for the administration of "liver" therapy

7 A low saturation index, as was first pointed out by one of us in 1926,^{*} strongly suggests that the anemia is due to chronic blood loss, and constitutes a definite indication for a search for the cause of the bleeding, an effort to stop the bleeding, and for the administration of "iron" in large doses

8 Anemia is rare in patients having malignant tumors without bone marrow metastases, hemorrhage or secondary infection. Hence, the finding of anemia in such patients should suggest that one or more of these complications has occurred

9 The color, volume, and saturation index determinations are extremely valuable aids in the differential diagnosis of anemias. It is necessary to warn, however, that they may be not only of no assistance, but actually misleading, if they are not determined by accurate methods with controlled technique and if the calculations are not based on the correct normal standards now available, instead of on the obsolete values still included in most texts^{*}

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CURRENT VIEWS ON THE ORIGIN AND MATURATION OF THE CELLS OF THE BLOOD*

CHARLES A. DOAN M.D., COLUMBUS, OHIO

MUCH of the accuracy in interpretation of the blood picture in disease is dependent upon an understanding of the underlying mechanism of hemopoiesis. The many techniques fixed vital and supravital which have been developed for the study of the cells of the blood together with data on cell origins and differentiation obtained from embryologic and experimental investigations now provide at least a working basis for the approach to the dyscrasias involving the hemopoietic system. Quite aside from the primary blood diseases, there are very few pathologic conditions with which the body has to deal in which one, or more of the types of cells represented in the circulating blood is not secondarily involved. That these circulating cellular elements may represent an important and powerful increment in the defense forces of the body is a fact attested by repeated observation. G. Lovell Gulland¹ in his Harveian Oration on the Circulating Fluid "looks forward to the time when the differential count will be more important than the auscultation of the heart," but that day will come in its fullness only when "the knowledge to interpret such data properly" is ours.

The bone marrow throughout extraterrene life is the natural source of erythrocytes, thrombocytes and the three kinds of granulocytes. The phagocytic macrophages (clasmatoocytes) while always present in marrow, are quite as regularly and normally found in spleen, liver and diffuse connective tissues. Their size and ability to arise in situ throughout the body, as the need presents tend to minimize their appearance in the circulation.^{2 3} Lymphocytes and monocytes, the remaining elements which utilize the vascular bed as an avenue of distribution, develop in lymph nodes, spleen, and in the more diffuse lymphoid and connective tissues of the body. Hence hemopoiesis in its broadest sense involves bone marrow, spleen, lymph nodes, connective tissues and the vascular and lymphatic systems.

Several circumstances have contributed to the confusion which has existed, and which still continues to prevail, among hematologists relative to the origins and relationships of the cells of the blood. It is agreed that all take their first beginning from the mesenchymal cells of the mesodermal layer in the embryo. But thereafter the theories and hypotheses diverge more or less radically though I would venture to assert that the differences arise more in the interpretation than in opposing objective observations where experiments have paralleled in materials and methods. In the usual hyperplastic red marrow where blood cells are developing it is difficult to distinguish the tissue landmarks which separate and segregate the units comprising different cell strains. This difficulty

*From the Department of Medical and Surgical Research, the Ohio State University.

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Several circumstances have contributed to the confusion which has existed and which still continues to prevail, among hematologists relative to the origins and relationships of the cells of the blood. It is agreed that all take their first beginning from the mesenchymal cells of the mesodermal layer in the embryo. But thereafter the theories and hypotheses diverge more or less radically though I would venture to assert that the differences arise more in the interpretation than in opposing objective observations, where experiments have paralleled in materials and methods. In the usual hyperplastic red marrow where blood cells are developing it is difficult to distinguish the tissue landmarks, which separate and segregate the units comprising different cell strains. This difficulty

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has been obviated by certain investigators through a study of less complex areas, either experimentally produced⁴ "or naturally occurring, as in the zone between red and yellow marrow, when hemopoiesis is extending." Under these conditions erythrogenesis may be observed to occur in foci separate and distinct from myelogenic centers and it is from analyses of such material that we have learned most about the relative location, distribution and independent origin of erythrocytes and granulocytes. One of the principal difficulties to an unanimous agreement upon these intercellular relationships resides in the fact that all of the cells of the blood continue to arise throughout life as primitive, immature elements incapable of performing their specific functions until after the completion of a definite maturation cycle. Most of the criteria characteristic of the definitive cells and which form the basis for morphologic differentiation and classification, are elaborated during this maturation period. Hence, the earlier precursors of each strain of cells lack in direct proportion to their immaturity, clear cut distinguishing features upon which hematologic investigators can agree. Furthermore the evidence obtained from tissue culture has tended to emphasize the importance of environment, and of particular food materials in modifying the morphologic characteristics of cells. This evidence has broadened the basis for the concept of cytologic dedifferentiation, and correspondingly diminished the probability of limited or highly specific potentialities, more particularly as applied to the lymphocyte.⁵ It is not strange, therefore, that many students of the subject (Maximow, Downey, Weidenreich, Dan-chakoff, Feriata) have concluded that one primitive, multi- or toto-potential lymphocyte or lymphocyte-like stem cell, the lymphoidocyte of Pappenhem, provides the sole ancestral background for all of the definitive blood cells now recognized. A consideration of this element must therefore, precede any consideration of the other blood cells.

THE LYMPHOCYTE

The typical lymphocyte of the circulating blood lacks the hemoglobin, the specific granules or the neutral red rosette, which characterize the erythrocyte, the granulocyte and the monocyte respectively. It thus lacks the more readily recognizable criteria of functional maturity. Moreover, the lymphocyte has never been demonstrated to perform any vital function comparable to that of the oxygen-carrying property of red blood cells, or the phagocytic defense or proteolytic enzyme actions of the neutrophilic leucocytes. The appearance of azur granules, or of an occasional neutral red vacuole, in the cytoplasm of the lymphocyte has only served to confuse rather than clarify the issue.

The extensive, and technically perfect, histologic studies of Maximow,⁶ including both embryonic and adult tissues, convinced him of the essential hemopoietic function of the "lymphocyte" which exists solely to be played upon by the various forces of the body, with the corresponding cellular differentiation a direct result of the need of the moment.

The Clarks¹⁰ have studied extensively the cytologic responses of blood and connective tissues in the web of the frog and more recently in the mammal by means of the ingeniously placed Sandison "window"¹¹ in the ear of the rabbit. While identifying monocyte and tissue macrophage as different phases of the

same cell type they have found no evidence of the transformation of the blood lymphocyte into other definitive forms

The Lewises¹² have studied the changes occurring in hanging drop preparations of blood from many species. They have followed the development of vacuoles in monocytes to the point where it was impossible to distinguish any morphologic difference between these changed cells and the naturally occurring highly phagocytic macrophages or classmatocytes of the *in vivo* tissues. "No such obvious transformation of lymphocytes was encountered," however.

Parker and Rhoads¹³ studied the cells removed from the circulation in cases of lymphoid leucemia after incubation for various periods of time. They observed that the lymphocytes "often showed some increase in the number and size of their neutral red granules which in arrangement frequently suggested a rosette. Such a rosette however could not be confused in any way with that of a monocyte owing to the shape and color of the granules."

Carrel and Ebeling¹⁴ found that the lymphocytes disappeared in their cultures of hen's blood and that only the "mononuclears" were transformed into macrophages and fibroblast-like cells.

Cunningham, Sabin and Doan¹ conclude from their experimental studies that a primitive stem cell for the white blood cells may be recognized in bone marrow lymph nodes spleen and the connective tissues. Fixed cells in the reticulum of these organs give rise by mitosis to a free primitive cell the size of the small lymphocyte. During times of increased demand for myeloid or lymphoid elements these small round cells may be found in increased number in association with the more mature cells of the respective strains. These studies differentiate this primitive stem cell from the lymphocyte, which latter was never observed by these investigators to develop into other definitive forms.

Jordan and his associates have studied hemopoiesis in the frog extensively over a period of years¹⁵ and they conclude that the differentiation of lymphocytes originating from reticulum cells provides the source for the various types of definitive blood cells in this species. Tischtschenko¹⁶ in a recent investigation of hemopoiesis published from his laboratory in Berlin concludes that the myeloid and lymphoid elements of the frog's blood "must belong unconditionally to two different hemocytogenic systems." Bloom¹⁷ cultured the lymph from the thoracic duct of the rabbit and found changes occurring which he interpreted as a metamorphosis of both large and small lymphocytes into typical inflammatory mononuclears, the so-called polyblasts of Maximow. Seeman¹⁸ working in Aschoff's Institute of Pathology at the University of Freiburg has found in studying the blood of the white rat that the "real lymphocyte from lymphatic tissue is not capable of transformation into monocytes and histiocytes" and states furthermore that "the extreme monophyletic school of Maximow places under the category of lymphocytes entirely different forms of cells which are only separated by supravital staining or biologic experiment."

Applying these two methods suggested by Seeman, Wiseman²⁰ has recently adduced some very pertinent facts bearing on this question of the identity of the lymphocyte. This investigator first analyzed all of the criteria which have been described as accompanying maturation in erythrocyte and granulocyte. Quite aside from the elaboration of specific materials in these better understood

strains of cells, which will be described later certain other significant changes occur. The dense basophilia of the cytoplasm common to all very young blood cells gradually disappears as hemoglobin or specific granules increase. The mitochondria, as revealed by supravital staining with Janus green, rapidly decrease in number with the approach of full maturity though in the earlier stages of development they are present in large number. The elaboration of chromatin material in the nucleus brings about a very definite change in the density of this structure from a young vesicular to a mature pyknotic state. With these criteria in mind, a superficial survey of the lymphocytes of the blood revealed differences in nuclear structure, basophilia and mitochondria strikingly similar to those noted in the other blood cells and which could quite readily be submitted to study and critical analysis. Wiseman analyzed these differences first in the normal adult, then in the newborn, and finally under conditions of experimentally induced, lymphoid, hypo- and hyperplasia in rabbits. He has classified the lymphocytes into three groups, according to the criteria cited. In the normal adult human being and the rabbit a relatively stable equilibrium in the proportion of 5 young, to 49 mature, to 46 old lymphocytes is maintained. As would be expected, if the criteria chosen really reflect the relative age of these cells, there was a marked "shift to the left" from this normal Y-M-O formula in the newborn and under conditions of lymph node hyperplasia. That is to say, Wiseman has recognized through these studies a maturation cycle for the lymphocyte of the circulating blood, based upon changing criteria identical with those which accompany the essential transformation of megakaryoblast to erythrocyte and of myeloblast to neutrophilic leucocyte, with a shifting of the Y-M-O index depending upon the state of activity of the lymphoid tissues. He has thereby established the lymphocyte, it would seem,—this enigma of the hemopoietic system—upon an equal and independent basis of existence with the other blood cells. A very distinctive type of motility for the mature lymphocyte is readily seen in living preparations of blood^{21 22} which contributes further circumstantial evidence, perhaps, toward its independent identity.

It should now begin to be apparent why the interpretations of equally competent and careful investigators have differed so widely up to and including the present time. Superficially, and even with the most meticulous care in analyzing fine cytologic differentiation on a morphologic basis alone, primitive basophilic cells destined to elaborate hemoglobin, or granules, or vacuoles partake of the undifferentiation, which marks to a large degree the entire life cycle of the blood lymphocyte. Only the most discriminating observation, and the submitting of these cells to such biologic experimentation and stimulation as involve an accentuation of their number and activity, can be expected to continue to further our knowledge and understanding of this strain of cells. Our present information, then, justifies both the position taken by those who have contended that "lymphocyte-like" cells may differentiate into other specific definitive cell types, and those who have seen no evidence of transformation in a large proportion of the small "mononuclear" round cells, which exist in blood and tissues. Neither hypothesis may be considered as mutually excluding the other.

Those who have used the supravital and fixed techniques extensively together, each for its respective unique contribution to the problem, feel that the early

precursors of the lymphocyte even the lymphoblasts may be distinguished from the "primitive stem cell" of the white cells and also from myeloblast and monoblast. The lymphoblast has a relatively deeper basophilia, larger rod-shaped mitochondria and fewer nucleoli (one to two) than do the leucoblasts of the other two strains of white cells. These differences may best be studied as exemplified in the "blast" cells of the acute leucemias myeloid, lymphoid and monocytic where the presence of more mature stages in the respective cell types clearly identifies those with less characteristic criteria. In this problem we have an excellent example of the contribution which the experimental study of physiologic function and of changing rather than static biologic form, may make to the science of pure morphology so frequently unappreciative of the aid which may be rendered by these handmaidens.

The implications of the fundamental studies of Wiseman which are now being applied to the study of the diseases involving the lymph nodes and lymphatic tissues give promise of a very real advance soon in our understanding of the different pathologic mechanisms underlying the dysfunctions affecting the lymphocytes.

THE GRANULOCYTE

As has been stated already the principal difference of interpretation with reference to the details of the mechanism of hemocytogenesis abides in the discussion as to just where if at all specificity and irreversible dedifferentiation finally begins. Before any specific granules have been elaborated there may be found in the liver and spleen of the embryo and in the bone marrow at later stages extravascular round cells, with a moderately basophilic cytoplasm many fine spherical mitochondria and a vesicular nucleus containing from two to five nucleoli these cells usually in close association with other extravascular units containing a few scattered granules. Partly because of their extravascular position in part because of their morphologic characteristics as outlined and in part through their association with definite myelocytes these units may be identified as myeloblasts. Though Naegeli, to whom we owe the original concept of the myeloblast does not limit the term to nongranular cells, it would seem best to reserve this designation for the agranular precursor of the myelocyte. An involved and highly technical nomenclature together with differing definitions for identical terms employed by the several schools of hematologic investigators have combined to surround this subject with an unnecessary mystery and complexity. Certain phenomena occur simultaneously and usually reciprocally during the period of maturation of the granulocyte and to comprehend the underlying facts in this metamorphosis is far more important than to be able to understand all of the terms which have been applied to the many minor morphologic variations which may be found among the myeloid cells wherever they are observed to be developing.

The primitive "lymphocyte-like" precursor of the myeloblast may be recognized most readily in relatively hypoplastic areas where myelopoiesis is actively extending. Under such circumstances mitoses may be observed occurring in the fixed cells of the intervascular spaces of the marrow reticulum situated similarly topographically to myeloid foci already functioning. The mitochondrial and basophilic content of the free cells thus formed increase to

a maximum just prior to the appearance of the first few specific granules. From this point on there is a rapid increase in the number of specific granules with a gradual disappearance of the mitochondria and basophilic substance. The only necessity for distinguishing various stages in this maturation period and christening them with names is to facilitate an appraisal of the acuteness of the process in a given case of myelogenous leucemia or severe infection, and to govern therapy. For these purposes the most accurate and dependable single criterion of maturity is the number of granules present in the cytoplasm of a given cell. The chemistry of the granules, basophilia, nuclear criteria, etc. are much more variable with respect to the ultimate criterion of maturity, which is active motility of the cell. Except under conditions of extreme toxicity, when an occasional motile neutrophile may be seen in the peripheral circulation with an incomplete complement of granules, a cytoplasm filled with granules plus motility are the essentially constant findings significant of functional maturity in this strain of cells.

But to provide means of statistically expressing the severity of the bone marrow insult or leucemoid hyperplasia in the patient it has been suggested that the phase of granule elaboration, the myelocyte period so called be arbitrarily divided into three stages: those cells containing ten or less specific granules by actual count may be designated Myelocytes A, when approximately one half the cytoplasm is filled with specific granules, mitochondria and basophilic material being still plentiful; this adolescence of the cell may be designated as Myelocyte B, just before the nucleus elongates preparatory to the formation of two lobes and before any evidence of motility is apparent, but yet with the cytoplasm filled with granules, we have what may be termed the most mature of the myelocytes, Myelocyte C.

As soon as the granulocyte becomes motile then except for the occasional vestige of those cytoplasmic criteria important during the myelocytic maturation, no further changes are noted except in the nuclei, until the final "non-motile" stage of Sabin²⁴ representative of the physiologic death of the cell. Aineh,²⁵ and later Schilling²⁶ and still later many others, have proved conclusively that the number of lobes in the nucleus of the neutrophile is the criterion of age for the mature leucocyte. Early and mild reactions affecting the myeloid function of the bone marrow may be detected by an increase in the percentage of those neutrophilic leucocytes having only two lobes or no lobing of the nucleus. A still further "shift to the left" and its degree, may be ascertained by partitioning the myelocytes according to the cytoplasmic criteria above mentioned; the study of the whole cycle thus providing data upon which to estimate quite accurately the state of myeloid activity in the bone marrow.²⁷

The various degrees of leucopenia which are now being recognized clinically, including the malignant neutropenia of Schultz²⁸ may be divided according to whether an actual deficiency of myeloid cells exists in the bone marrow or whether other factors have combined to produce temporarily a lowered count in the blood stream, with the potential source of supply still intact.²⁹ The rationale of nucleotide therapy³⁰⁻³² in neutropenic conditions must depend upon a very careful differential diagnosis of the underlying mechanism responsible for the finding.

The maturation cycles of eosinophile and basophile vary in no particular from that described for the neutrophile the earliest granules in these cells showing the special morphologic and staining characteristics so well known in the respective definitive cells

No longer is it sufficient to know only the total white cell count and the relative proportion of granulocytes lymphocytes and monocytes present if the maximum of information is to be secured in the clinical appraisal of the patient We have already indicated the increased value which may attend the additional qualitative study of the granulocytes and lymphocytes and it can be said that this general principle is in no wise excepted when it comes to the study of the monocyte

THE MONOCYTE

Until very recently the nongranular cells of the blood were all classified together as lymphocytes the transitional or large mononuclear of Ehrlich, or, as it is now more frequently designated the monocyte receiving little attention from physicians When stained with any of the Romanowsky dye combinations, the monocyte of human blood usually shows a myriad of tiny azurophilic granules studded in a background of mottled blue cytoplasm Because of this finding it was thought originally to represent a transitional stage between lymphocyte and granulocyte Later when Michaelis and Wolfe³¹ demonstrated so-called azur granules in certain of the lymphocytes the monocyte was more closely linked in the minds of many with the lymphocyte Schilling³² was the first to quite definitely and finally classify it as a separate entity within the group of white blood cells The studies of the past decade have now made it very clear that many of our most important interpretations in clinical medicine are dependent upon the accurate identification of monocyte and lymphocyte in the differential count

An appreciation of the morphologic characteristics physiologic function and pathologic potentialities of the monocyte has followed close upon the development of the supravital staining technic³³ and the modern experimental and clinical study of tuberculosis³⁴ Sabin Doan and Cunningham³⁴ described a rather characteristic rosette of neutral red vacuoles surrounded by mitochondria which are not apparent in the ordinary fixed preparations The arrangement and behavior of these vacuoles together with a very distinct surface-film type of motility in living preparations have served to differentiate sharply the monocyte from the lymphocyte

That the monocyte is closely related to the "primitive cell" of the connective tissues similar in all morphologic and other characteristics to the primitive cells of the lymph node and bone marrow has been clearly demonstrated Doan and Sabin³⁵ noted the appearance of large numbers of monoblasts devoid of vacuoles in the bone marrow of rabbits experimentally infected with bovine tuberculosis These cells soon developed the typical rosette of neutral red staining vacuoles and were then rapidly transformed into typical mono- and multinucleated epithelioid cells arranged in tubercles The same sequence of events has been observed in the cytologic proliferation occurring in the various organs and tissues of the body during the development of a generalized tuberculous infection

Folkner³⁶ has studied particularly the normal formation of monocytes within the peripheral nodes of normal rabbits, and has described and effectively illustrated, large, young monoblasts, premonocytes or early monocytes showing the beginning development of vacuoles and the mature monocyte with its fully developed rosette all within one focus, quite as we find myeloid foci with cells in various stages of maturity in the bone marrow.

Hyperplasia of monocytic tissue is thus quite as possible and frequent an occurrence as myeloid or erythroid hyperplasia, and, when it occurs, is reflected quite as quickly and directly, qualitatively as well as quantitatively, in the peripheral circulation. The monocyte-lymphocyte ratio is of distinct prognostic significance in tuberculosis, the quality of the individual cells in each of the categories being equally important. While tuberculo lipoids have a very strong irritative or stimulative effect upon the monocytes very many other substances also call forth this response in a greater or lesser degree, and many types of general tissue reactions involve the monocytes.

THE TISSUE MACROPHAGE OR CLASMATOCYTE

In the first section of this discussion, it was made apparent that no unanimity of opinion exists at the present time relative to the monocyte-clasmatocyte question. Most of the evidence from tissue culture tends to support the contention that they represent two phases in the life history of the same cell type. While recognizing the occasional highly phagocytic monocyte as approaching very closely, and possibly, rarely, entirely simulating, the tissue macrophage in histologic appearance, nevertheless, the great majority of the phagocytic cells may be very readily separated into two groups in the supravital studies after various experimental procedures. Different pathologic processes strikingly call forth one or the other in rather characteristic predominance, though both are, of course, frequenters of all the organs and tissues.

The large phagocytic cells filled with disintegrating erythrocytes in spleen, lymph nodes, and bone marrow, and the Kupffer cells of the liver sinuses appear to be closely related to endothelium. In the sinuses of lymph nodes, which are draining an area of hemorrhage or necrotic debris, the endothelial cells engulf in situ the foreign material to such an extent that in many places the sinus endothelium will have been entirely denuded and the free, rounded up, highly phagocytic endothelial cells will be free in the tissues indistinguishable from those in marrow and spleen normally. Whether there is also an extravascular origin for cells with this very highly specialized scavenger activity remains problematical and unsettled up to the present time. Perhaps, we may assume a three-fold source of these tissue macrophages on the basis of the evidence available, from endothelium, from monocytes, and from preexisting clasmatocytes.

Only rarely do these cells find their way into the peripheral capillary circulation^{2, 3} probably because of their large size and inelasticity, when highly phagocytic.

THE BLOOD PLATELETS

Both direct and inferential evidence has continued to accumulate during the past quarter of a century, attributing to the megakaryocyte of the bone

marrow the origin of the blood platelets. The unsatisfactory state of the technical procedures for estimating the total number of these bodies is revealed in the multitude of methods, which flood the literature and the wide range in the figures usually reported. It, thus, becomes important to analyze these essential blood elements qualitatively to supplement and strengthen the weakness inherent in the attempt to determine their quantity.

Apparently these smallest of the formed elements of the blood behave very much like the larger blood cells already discussed. When the platelets are markedly reduced in number under pathologic conditions, there is variation in size with a relative increase in the smaller forms. In addition there is a varying degree of basophilic staining of the cytoplasm with irregular distribution of the tiny granules. When the count is high or when the platelets are rapidly increasing in number they tend to be larger and less granular. In the period of rapid increase which reflects a rapid multiplication of megakaryocytes in the marrow, basophilia is common. Basophilia of the platelet cytoplasm indicates immaturity³⁷ as it does in all of the blood cells.

THE ERYTHROCYTE

The erythrocyte is the first of the blood cells to appear in embryonic development. Following the extensive studies of fixed embryonic material by Maximow³⁸ and Danekofsky³⁹ Sabin⁴⁰ studied the living chick blastoderm of the second day of incubation observing the differentiation of the first angioblasts from the primitive mesoderm. These angioblastic nests were then seen by her to give rise to the first blood plasma through liquefaction of some of the central cells. Those on the periphery elongated to form the first vascular endothelium, and the remaining cells comprised the original blood islands of erythroblasts appearing in the area pellucida. Subsequently, Sabin observed the endothelium giving rise to free hemoglobin synthesizing units.

Erythrogenesis ordinarily occurs in the adult in relatively hyperplastic areas of the marrow. It has been necessary, therefore, to analyze relatively hypoplastic states, where myeloid and erythroid foci were widely separated, and not too numerous. This has now been accomplished in both the experimental animal⁴¹ and in studies of selected human material⁴² secured at biopsy or postmortem.

In the first place it was found that there exists in marrow an extensive capillary system⁴³ most of which usually is collapsed and nonfunctioning as a patent vascular bed but which potentially is capable of marked hemopoietic activity. Marked hypertrophy and hyperplasia of the endothelial cells lining these "intersinusoidal capillaries" precedes the appearance of the first cells with hemoglobin. The megaloblasts while partaking of the relative lack of differential criteria noted in all young blood cells in fixed preparations still are characterized by a faint blush of hemoglobin when observed in the living state and possess a rather distinctive vesicular nucleus with one large nucleolus very occasionally two. In areas of rapidly extending erythrogenesis endothelial mitoses are numerous and the first megaloblasts are to be found within the capillary network closed to the active circulation. Small isolated islands of erythroblasts develop from these first megaloblasts and on serial section can be demon-

striated" ⁴¹ within endothelial lined channels which communicate with the sinusoids directly. By the gradual elaboration of hemoglobin at the expense of a decreasing basophilia and mitochondrial content the stage of the normoblast is reached. The main reserve of the red cells in marrow is held under normal conditions at this normoblastic level. ⁴² Extrusion of the pyknotic nucleus precedes delivery of the mature erythrocyte through the conical opening of the erythrocytic capillary into the active circulation. Reticulation or some other manifestation of remaining cytoplasmic basophilia, such as polychromatophilia is the only evidence of youth which can be recognized in the mature mammalian erythrocyte. The percentage of reticulocytes per cubic millimeter of blood is a measure of the rate of delivery of these units to the circulation.

Once again recurs the difference in interpretation of evidence which has marked all of the discussions centering about the origin and maturation of the cells of the blood. Does the erythrocyte take its origin from a multipotential "lymphocyte" under the stimulus of a favorable environment, or, given the propitious circumstances must there also be a different stem cell with a more specific capacity for synthesizing hemoglobin? The relationship which seems to exist between endothelium and erythropoiesis in early embryonic life is the logical point of approach to this problem in the adult. The evidence of endothelial activity, which always accompanies erythropoiesis in the bone marrow of the adult mammal particularly when taken in conjunction with the data available from embryologic studies would seem to have more significance than that of a mere fortuitous circumstance. However it is the opinion of many students of this problem that a free hemoblast "lymphocyte" located extravascularly in the marrow parenchyma is the precursor of the normoblastic nests of cells, which when mature either pass through fenestrations in the vascular endothelium, or if, as most investigators now believe the vessels of the marrow form a closed circulation, erode temporarily by "growth pressure" ^{44 4} an opening sufficient to permit entrance of the erythrocytes into the blood stream.

The perplexing questions concerning the place and mode of origin and the mechanism of delivery of the definitive erythrocytes have no direct bearing at the present time upon the practical clinical implications. The sequence of events in the maturation cycle is quite clear and this knowledge provides the clinical pathologist with a basis for his analysis of the various syndromes involving the red blood cells, a study which must always precede the institution of rational therapy in the individual case. The hypoplastic and aplastic anemias are the result of deficient megaloblastic differentiation. In pernicious anemia the marrow is hyperplastic but the megaloblasts for the most part lack the power to mature, to elaborate hemoglobin. All of the many types of secondary anemia involve a deficiency in the ability to utilize or in the supply of iron and other elements, essential to hemoglobin formation.

Greater advances have been made during recent years in our understanding and control of the anemias than in the diseases involving primarily the other formed elements of the blood. The increased interest, which is centering about the cells of the blood, as they affect both directly and indirectly the general health of the individual, ⁴⁶ gives promise of a continually improving therapeutic rationale in our attack upon disease.

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THE SIZE AND HEMOGLOBIN CONTENT OF THE ERYTHROCYTE*

METHODS OF DETERMINATION AND CLINICAL APPLICATION

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THE fact that even today relatively few physicians interest themselves in details concerning the size and hemoglobin content of red corpuscles in attempting to differentiate the anemias, is surprising when one finds that some of the most important physical characteristics of the erythrocyte and their variation in disease have been known almost since the first days of the microscopic study of blood. Welcker, who with Vierordt recorded the earliest erythrocyte counts, determined the volume of the red corpuscle with the aid of plaster models. He found (1864) that the blood corpuscles in a case of chlorosis were smaller than normal. Johannes Duncan about the same time (1867) recognized the possibility of variation in size and hemoglobin content of red corpuscles and Sørensen (1876), Ehrlich (1880), and Laache (1883), noted the increase in the size of the cells in Addison-Biermer (pernicious) anemia. Havem (1878) measured the diameter of red corpuscles, calculated their mean volume from these measurements, and devised the color index.

There can be little doubt that these determinations with the exception of the color index, failed to arouse the enthusiasm of the general medical public as much because of technical difficulties as on account of general ignorance concerning the value of such examinations. The earlier hematocrits, such as those of Hedin (1890) and Daland (1891), could not be depended on for accurate and consistent information and even instruments developed more recently such as the Van Allen hematocrit (1925), have by no means satisfied critical workers. Although the determination of the average amount of hemoglobin in the red corpuscles (color index) has been very commonly carried out, the notorious inaccuracy of hemoglobin estimation, so general even today, has brought this constant into considerable disrepute. The introduction of accurate methods, such as those for the measurement of cell diameter utilized by Price-Jones²⁰ and others,^{1, 2} has likewise not been followed by the universal enthusiasm which the information derived may deserve because of the time-consuming and tedious nature of these studies. There is little question that general interest in the study of the size and hemoglobin content of the erythrocyte, in spite of proof of the importance of these observations, can only be aroused by the presentation of simple and accurate methods.

METHODS

No attempt will be made in this paper to present completely all the methods which are available for the determination of the size and hemoglobin content of the red corpuscles. Instead it is proposed to mention only briefly those methods

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which are of historical importance or are of relatively little value and to describe fully those which in the writer's own experience are most useful from the standpoint of simplicity and accuracy.

Measurement of Erythrocyte Diameter—Although the diameter of red corpuscles was measured in the very early days of hematology, it was not until ten years after the first of the painstaking studies of Price Jones was published (1910) that considerable interest was aroused in these determinations. Price Jones projected the images of the red corpuscles on a sheet of paper, and after outlining in pencil the cells in a thin portion of the smear, measured their long and short diameters. The magnification of the cell images being known, it was possible to calculate the actual size of each cell. The results of the measurement of five hundred cells were plotted in the form of a curve which has come to be known as the 'Price Jones curve'. The method of Price Jones although quite accurate, is obviously too time-consuming for clinical laboratory work. Various modifications have been recommended, the simplest being the following:

A micrometer disc of glass marked with a scale, the smallest division of which equals approximately one micron is placed in the eyepiece of the microscope. Since tube length and lens differ in various microscopes, it is necessary to calibrate the micrometer disc for the tube length and lenses employed. This is very easily done with the aid of a micrometer slide specially made for this purpose, or a hemocytometer may be used instead. The micrometer disc having been placed in the eyepiece of the microscope, the hemocytometer is focused and adjusted in such a way that the divisions of the micrometer disc are superimposed over one of the smallest divisions of the hemocytometer. Since the latter are exactly fifty microns to the side, it is simple enough to determine how many of the micrometer divisions make up fifty microns, and from this to calculate the width of one division.

Fresh blood or fixed and stained smears may be employed but it is important to bear in mind that a uniform technic must be followed. Fixing and drying of red corpuscles cause shrinkage in their size.³⁰ Smears should be well stained and thin. There is some variation in the values reported for normal mean erythrocyte diameter (Price Jones, 72 μ ; Ghosh and Stifel, 74 μ ; Bell et al, 77 μ ; Wiechmann and Schumeyer 79 μ). The cause of these differences is not clear but since they may be due to slight differences in technic, it is advisable to establish the normal for one's own working conditions by examining several specimens of normal blood before carrying out any series of determinations in disease.

The actual measurement of the cells is carried out by bringing the erythrocytes in a thin portion of the preparation under the scale of the micrometer disc. The maximum and minimum diameters of each cell may be measured and the mean of these taken as the diameter of the erythrocyte, or only one diameter of all round cells which appear under the scale may be recorded. It is obvious that greater accuracy is secured by measuring two diameters of each of a large number of cells. Usually several hundred red corpuscles are examined in this way. The mean diameter of all the cells is calculated, but it is also important to note the degree of scatter (the proportion of microcytes and macrocytes) for,

as Price-Jones has emphasized the scatter may be such that the mean cell diameter is normal or nearly normal. Scatter is best demonstrated by graphically recording the number of erythrocytes of each size encountered (Price-Jones curve).

Comparative Value of Methods for Determination of Cell Size—The measurement of cell diameter gives quantitative expression for what one sees on examining a blood smear. This information is very valuable but from a practical standpoint must be measured in terms of the time and effort required and the possibility of deriving similar information by simpler methods. Various instruments have been devised by means of which cell diameter may be measured much more easily than by the technic above described. These methods (diffraction methods of Pijper, Millai, eyemeter of Emmons, halometer of Eve) are based on the principle that circular concentric spectra the character and size of which depend on the size and shape of the red corpuscles are produced when parallel light rays are passed through a blood smear placed in front of a convex lens. The very important disadvantage of all these methods is that the size of the corpuscles is measured in mass and only mean diameters are thereby determined. Variability cannot be accurately measured. As Price-Jones himself admits, variations in cell diameter are relatively so small and of such variety in disease, that mean cell diameters may often not be significantly altered from the normal. Of thirty-two determinations in 14 cases of pernicious anemia and 5 cases of spina, I found mean cell diameter greater than the maximum normal in only 15 instances and even in 6 of these the values were little greater than the normal.⁴⁰ On the other hand, Haden¹⁶ and I⁴⁰ have shown that it is possible to demonstrate striking differences in mean values when the average volume of the red corpuscles is determined, for, in measuring volume small variations in size in all dimensions are taken into account and thereby the differences are magnified. For this reason as well as from the standpoint of accuracy the measurement of mean cell diameter by one of the diffraction methods is inferior to the determination of mean cell volume by the method to be described. With regard to the measurement of individual cell diameter as is carried out by means of the micrometer disc no criticism can be offered except that which derives from the tedious and time consuming nature of the procedure. However the latter objection is most important from the standpoint of general practicability. In my own experience the simple examination of a blood smear together with the measurement of mean corpuscular volume has afforded considerably more useful and accurate information at the expense of much less effort than is involved in measuring the diameter of several hundred red corpuscles. The hemitocrit method to be described possesses the additional advantage that in combination with hemoglobin determinations valuable information concerning the concentration of hemoglobin in the red corpuscles may be gained which can not be derived in any other way.

Measurement of Corpuscular Volume—There is no practical method by means of which the volume of individual red corpuscles may be determined. It is only possible to determine the mean corpuscular volume of a substantial number of cells. This is done by determining the volume of packed red corpuscles as well as the number of cells in a given quantity of blood and from these values

the volume of the average erythrocyte is calculated. The volume of packed red cells may be determined by centrifuging a quantity of blood to which a suitable anticoagulant has been added. Centrifugation is carried out until no further packing occurs.

Hematocrits are available which require only such small quantities of blood as may be obtained by finger puncture,^{1 32} but since greater accuracy is afforded by the use of larger quantities of blood, venipuncture is recommended. When care is taken to avoid congestion of the arm from tourniquet pressure, no difference is found between venous and capillary blood. It is our custom to prepare the needle and syringe, clean the arm for venipuncture, and fasten the tourniquet just prior to making the puncture. When this routine has been followed, blood counts on capillary and venous blood have been found to agree exactly. Venous blood so obtained may be used not only for hematocrit determinations but also for red cell counts, hemoglobin, leucocyte, platelet, reticulocyte, and other hematologic procedures. There is the additional advantage that counts may be checked without the necessity of troubling the patient more than once.

Heparin is the ideal anticoagulant, since it does not affect the size of the red corpuscles but it cannot always be depended upon unless the extremely expensive, purified material is employed. The use of an isotonic anticoagulant, such as 1.4 per cent sodium oxalate solution, has the obvious disadvantage that the blood is diluted and a source of error is introduced not only in hematocrit determinations but also in any blood counts which it may be necessary to carry out. For these reasons, dry potassium oxalate in minimal amounts, namely, 10 mg per 5 cc of blood, is preferred. This quantity is easily measured by running 0.5 cc of 2 per cent potassium oxalate solution from a burette into a vial or small bottle and allowing the water to evaporate. In the proportion of 2 mg per cc of blood, solid potassium oxalate, does not influence red cell, leucocyte, platelet, or other counts but causes a shrinkage of 8.2 per cent in the volume of the red corpuscles as compared with their volume in heparinized blood.*

For the hematocrit, I have found most useful a glass tube of about 2.5 mm uniform inside bore and flat inside bottom. On the sides of this instrument,^{36 †} a centimeter-millimeter scale 10 cm in length is etched. About 0.7 cc of blood is required. The hematocrit is easily filled by means of a capillary pipette. When the leucocyte count is high, it is advisable to allow the filled hematocrit to stand for an hour or longer before centrifugation, in order to permit separation of the red and white corpuscles. Centrifugation at 3,000 revolutions per minute for a half hour is necessary to secure complete packing. It is not necessary, however, to determine the speed of the centrifuge for, since the object of centrifugation is to secure complete packing, it is simple enough to determine the time necessary for any instrument even though its speed is not known.

In order to deal with values which may be readily visualized, it is preferable to calculate the *mean corpuscular volume* in cubic microns. This is done by di-

*The volume of packed red cells as determined from blood to which has been added solid potassium oxalate in the proportion of 10 mg per 5 cc of blood should be multiplied by the factor 1.09 in order to correct for shrinkage.

†The hematocrit may be obtained from Arthur H. Thomas Co. Philadelphia or Will Corporation, Rochester, N. Y.

viding the volume of packed red cells expressed in cubic centimeters per 1000 cc of blood by the number of red cells expressed in millions per cubic millimeter. The result gives the average volume of the red corpuscles in the sample of blood in cubic microns. Thus for a sample which contains five million red corpuscles per c. mm. and 425 cc. of packed red cells per 100 cc. of blood the mean corpuscular volume is $425 \div 5.0 = 85 \text{ c. } \mu$.

Objections have been raised against this method of determining corpuscular volume. Some observers have thought that by centrifuging fluid is forced out of the blood corpuscles, thus making their volume smaller. This effect is unlikely because the volume of the blood corpuscles is determined by osmotic forces which are so great that the force with which they are centrifuged down is negligible in comparison. That it does not take place is shown by the fact that the mean corpuscular volume of normal blood as determined by the hematocrit method here described agrees almost exactly with the value obtained by Ponder and Saslow²⁷ by an accurate (but laborious) colorimetric method.

From a detailed study of methods of measurement of red cell volume, Ponder and Saslow²⁷ concluded that the hematocrit method is inaccurate. They used as hematocrits capillary tubes 100 mm. long and centrifuged three sets of these tubes at speeds of 1000, 4000 and 14000 r.p.m. Spinning was carried out to the attaining of constant volume or Koeppe's criterion. They could find no consistent agreement with the results of volume determination obtained by their colorimetric method. It seems likely, however, that the hematocrit technique employed by these investigators is at fault rather than the hematocrit method as a whole, for the values for mean corpuscular volume obtained by the method described by the writer not only agree almost exactly with those of Ponder and Saslow as determined by what they consider a much more accurate method, but the same results are consistently obtained. In Table I will be found the results of four experiments which were carried out to determine the probable error of

TABLE I
PROBABLE ERROR OF DETERMINATION OF VOLUME AND HEMOGLOBIN CONTENT OF RED CORPUSCLES

EXP. NO.	MEAN CORPUSCULAR VOLUME		MEAN CORPUSCULAR HPG		MEAN CORPUSCULAR HGB CONCENTRATION	
	STANDARD DEVIATION	COEFFICIENT OF VARIATION	STANDARD DEVIATION	COEFFICIENT OF VARIATION	STANDARD DEVIATION	COEFFICIENT OF VARIATION
1	1.04 c. $\mu \pm 0.25$	1.08%	0.33 $\gamma\gamma \pm 0.07$	0.97%	0.33% ± 0.08	0.93%
2	0.48 c. $\mu \pm 0.11$	0.65%	0.43 $\gamma\gamma \pm 0.09$	2.08%	0.23% ± 0.06	0.82%
3	2.02 c. $\mu \pm 0.48$	1.63%	0.52 $\gamma\gamma \pm 0.12$	1.33%	0.23% ± 0.05	0.72%
4	0.03 c. $\mu \pm 0.01$	0.04%	0.02 $\gamma\gamma \pm 0.01$	0.13%	0.37% ± 0.08	1.46%

the methods followed in our laboratory. In each experiment the following procedure was repeated five times on the same sample of venous blood: (1) two red cell counts were carefully made, using two dilutions (the average of the two was employed in the calculation), (2) the hemoglobin was determined by means of a Newcomer hemoglobinometer which had been restandardized by the Van Slyke method, and (3) the volume of packed red cells was determined, using

a special hematocrit¹⁶ and an International Centrifuge (Size 1 type SB head 9 cm) rotated at 3,000 r.p.m. for thirty minutes. The small error clearly demonstrates the reliability of the results.

Determination of the Hemoglobin Content of the Erythrocyte—If in addition to determining the number of red cells and the volume of packed erythrocytes in the sample the hemoglobin be determined as well it is possible by simple calculation to derive valuable information concerning the hemoglobin content of the red corpuscles. This is an important advantage of the technique here described. As in the case of the red cell counts and hematocrit determinations accuracy is of course fundamental. We employ a Newcomer hemoglobinometer which has been restandardized by the Van Slyke method. Hemoglobin is determined in grams per 100 c.c. of blood.

The *mean corpuscular hemoglobin* or average amount by weight of hemoglobin in the red cells is determined by dividing the amount of hemoglobin, expressed in grams per 1000 c.c. of blood by the number of red cells, expressed in millions per cubic millimeter. The result gives the average weight of hemoglobin in the cells in micromicrograms.*

The *mean corpuscular hemoglobin concentration* or average concentration or saturation of the red corpuscles with hemoglobin is determined by dividing the amount of hemoglobin expressed in grams per 100 c.c. of blood by the volume of packed red cells expressed in cubic centimeters per 100 c.c. of blood and multiplying the result by 100. Mean corpuscular hemoglobin concentration is expressed in per cent. It will be noted that its calculation is similar to the calculation of the strength of any solution. Although the implied presumption that hemoglobin is contained in the red cell in the form of a solution is probably incorrect, the determination of this relationship of amount of hemoglobin to size of cell is nevertheless very valuable, as will be shown later.

TABLE II
METHOD OF CALCULATION OF CORPUSCULAR CONSTANTS

MEAN CORPUSCULAR VOLUME (CV)	Volume of packed red cells (m.c.c. per 1000 c.c. blood)
in cubic microns (c μ)	= $\frac{\text{RBC (in millions per c. mm.)}}{\text{Hemoglobin (in gm per 1000 c.c. blood)}}$
MEAN CORPUSCULAR HEMOGLOBIN (CH)	Hemoglobin (in gm per 1000 c.c. blood)
in micromicrograms ($\gamma\gamma$)	= $\frac{\text{RBC (in millions per c. mm.)}}{\text{Hemoglobin (gm per 100 c.c. blood)}}$
MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION (CC)	$\frac{\text{Hemoglobin (gm per 100 c.c. blood)}}{\text{Volume packed RBC (c.c. per 100 c.c. blood)}} \times 100$
in per cent (%)	

The method of calculation may be illustrated by the following:

A sample of blood contains 5.0 million red blood cells per cubic millimeter 14.5 gram of hemoglobin per 100 c.c. of blood and 42.5 c.c. of packed red cells per 100 c.c. of blood. Then

*A micromicrogram is the millionth millionth part of a gram or gram $\times 10^{-11}$ and is abbreviated $\gamma\gamma$.

$$\text{mean corpuscular volume (C V) is } \frac{425}{50} = 85 \text{ c } \mu$$

$$\text{mean corpuscular hemoglobin (C H) is } \frac{145}{50} = 29 \text{ } \gamma \gamma$$

$$\text{mean corpuscular hemoglobin concentration (C C) is } \frac{145}{425} \times 100 = 34\%$$

It will be evident that these calculations are similar to those used for the calculation of the volume color and saturation indexes respectively differing from the latter only in that absolute instead of relative values are employed. This method is preferable to the calculation of the indexes for the following reasons:

1 The number of red cells and volume of packed red cells for any sample of blood are always determined in absolute terms. In order to calculate the indexes it is necessary to convert these values into terms of per cent of normal. This is not required for the calculation of the constants here recommended. Again although hemoglobin is recorded in per cent by many there is a growing number of critical physicians who appreciate that it is more accurate to express hemoglobin directly in grams for the reason that the use of widely differing standards of normal is thereby avoided. Hemoglobin values expressed in absolute terms (grams) require no conversion into per cent for the calculation of the corpuscular constants.

2 In order to convert the red cell count, hemoglobin and volume of packed red cells to terms of per cent of normal, it is necessary to adopt standards of normal. That such standards must necessarily be arbitrary and erroneous is obvious when one considers the wide fluctuations in normal values associated with differences in sex, age and possibly also geographical location.²⁷⁻³¹

3 Whereas the indexes express only relative values the corpuscular constants afford absolute information concerning the erythrocyte and permit the visualization of the physical state of the red cell.

CLINICAL APPLICATION

Normal values for the size and hemoglobin content of the erythrocyte are recorded in Table III. The values for cell diameter must be considered as somewhat approximate for as already mentioned there are slight differences in the

TABLE III
NORMAL VALUES FOR SIZE AND HEMOGLOBIN CONTENT OF ERYTHROCYTES

	AVERAGE	MINIMUM	MAXIMUM
Mean Corpuscular Volume (C V) in cubic microns	87	80	94
Mean Corpuscular Hemoglobin (C H) in micromicrograms	29.5	27	32
Mean Corpuscular Hemoglobin Concentration (C C) in per cent	35	32	38
Mean Corpuscular Diameter in microns	7.5	6.7	8.0

diameter of the red cell as recorded by various observers. No significant differences in the size or hemoglobin content of the red cells in regard to sex have been noted, but it is now well known that the cells of the newborn are larger than those of adults.³⁷ Price Jones^{20, 36} and others have observed some increase in the diameter of red corpuscles during the day and as the result of violent exercise. However, neither Divenie et al.⁶ nor Ponder and Saslow⁸ have been able to confirm these results, nor have Haden¹⁶ or I (unpublished data) observed similar changes in the volume of the red cell. Likewise, I could find no relation between the size and hemoglobin content of the red corpuscle and the biologic reference—flame represented by body-weight, stature or surface area of men and women.³⁸ In general, the erythrocyte may be regarded as being remarkably constant in its physical characters and an index of an internal environment regulated to great constancy in the normal individual. This is in striking contrast to the variations observed in disease.

Although Welcker (1864) observed that in chlorosis the red corpuscles are smaller than normal and Sørensen (1876) pointed out that in Addison-Biermer anemia they are abnormally large, it remained for Capps in 1903, to stress the fact that significant alterations in the size of erythrocytes occur in anemia and that these changes are the result of variations in the growth, development, and destruction of the cells. In 1910 Larabee made similar observations but until the last few years interest in this subject has been only spasmodic.^{3, 12, 13, 14, 16}

In association with certain diseases there arise differences in the size and hemoglobin content of red corpuscles, the recognition of which is not only of assistance in diagnosis, but through more accurate differentiation gives important information concerning prognosis and therapy. Not only is it possible by the special hematologic methods here described to recognize pernicious anemia and the other macrocytic anemias but there may readily be distinguished a type of anemia which has hitherto rarely been differentiated from the large group of so called "secondary" anemias.

It is possible on the basis of differences in the size and hemoglobin content of the erythrocyte to distinguish 4 types of anemia.³⁸ These are

- 1 Macrocytic anemias, characterized by an increase in the mean volume and hemoglobin content of the erythrocytes and represented chiefly by pernicious anemia and many cases of sprue.

- 2 Normocytic anemias, which are distinguished by the presence of red cells of normal size and hemoglobin content. This group includes cases of anemia resulting from acute blood loss, hemolytic anemias (malaria), and aplastic anemias.

- 3 Simple microcytic anemias, which are characterized by a moderate reduction in the size of the red cells with no or little reduction in their hemoglobin concentration. In this group are found the majority of the anemias associated with chronic infections and toxic processes.

- 4 Hypochromic microcytic anemias. In this type of anemia there may be little or no reduction in the red cell count. Nevertheless the red corpuscles are found to be very small and are poorly filled with hemoglobin.

The determination of the size and hemoglobin content of erythrocytes is chiefly important in that it makes possible the ready differentiation of the macrocytic and the hypochromic microcytic anemias from the other two types.

Macrocytic Anemias—For many years physicians have largely depended on the color index for the recognition of macrocytic anemias. It is common knowledge that in pernicious anemias the color index is greater than 1. It is less generally appreciated that this does not mean that the red cells are hyperchromic or supersaturated with hemoglobin, an erroneous conception to which the use of the color index has led. By the calculation of the size and hemoglobin content of the erythrocyte in absolute terms a clear conception of the state of the red corpuscles in this disease may be gained. These calculations indicate that the average red cell in pernicious anemia contains a greater amount of hemoglobin than is normal (mean corpuscular hemoglobin is high) but this increase in hemoglobin content is no greater than the increase in size so that the concentration of hemoglobin in these cells (mean corpuscular hemoglobin concentration) is normal and may even sometimes be lower than normal.

The mean corpuscular volume is consistently found to be high in pernicious anemia in relapse. Of 56 cases examined at various stages of relapse and incomplete remission the mean corpuscular volume was greater than $95 \text{ c } \mu$ in 50, and greater than $90 \text{ c } \mu$ in 54. Of 37 of these cases in which the red cell count was less than three million, mean corpuscular volume was greater than $100 \text{ c } \mu$ in 30, between 95 and $100 \text{ c } \mu$ in 2 and between 90 and $95 \text{ c } \mu$ in 3. The mean corpuscular volume was lower than $90 \text{ c } \mu$ in 2 cases. In one of these transfusion had just been carried out. Subsequent determinations in the other case showed significantly high values. In no case was the mean corpuscular volume lower than normal. The values for mean corpuscular volume ranged as high as $164 \text{ c } \mu$, but usually were found between 100 and $125 \text{ c } \mu$. Although frequently the volume of the cells was greater in those cases in which the anemia was most severe this was by no means always true. The variations in corpuscular volume seem to be related to the nature and extent of red cell formation and destruction, and especially to the reticulocyte response (unpublished data).

One of the most important uses of the determination of mean corpuscular volume is in the recognition of cases of pernicious anemia in which the anemia is slight or moderate in degree. It is well known that at such a stage there may be considerable difficulty in diagnosis especially in those cases in which neurologic manifestations are present when confusion with other diseases is not unusual. When the red cell count is greater than three million the blood smear may not be of much assistance in diagnosis and measurement of the diameter of the red corpuscles may afford results which must be interpreted as normal or so nearly normal that little help in diagnosis is gained. Here the measurement of mean corpuscular volume by taking into account changes in all dimensions and thereby magnifying many small differences finds one of its most important uses. Haden has pointed out that an increase of 1 micron (13 per cent) in the diameter of a red cell is associated with an increase of 44 per cent in its volume. In Table IV are recorded the first blood examinations in 19 cases which were subsequently proved to be pernicious anemia and in which the red cell count was greater than three million. It is interesting to observe that the magnitude of the corpuscular volume was correlated with the proportion of the red cell count to the normal value for the sex of the patient rather than to the actual value of the count. The cases in which the red cell count was from three to four

TABLE IV

SIZE AND HEMOGLOBIN CONTENT OF RED CORPUSCLES IN CASES OF PERNICIOUS ANEMIA WITH MODERATE OR LITTLE ANEMIA

SEX	RBC		CV c μ	CH γγ	CC %
	NO $\times 10^6$	% OF NORMAL			
F	3.21	62	109	38	35
M	3.79	69	107	34	31
M	3.89	71	106	36	34
F	3.57	75	109	40	36
M	4.11	75	103	37	36
F	3.60	75	98	34	35
F	3.66	77	92	32	35
M	4.37	79	100	39	39
M	4.48	81	98	32	33
M	4.54	83	88	32	36
F	4.02	84	84	30	35
F	4.03	84	96	32	33
F	4.17	87	98	34	34
F	4.22	88	98	33	34
F	4.23	89	90	33	37
F	4.24	89	86	29	34
F	4.28	89	94	29	34
F	4.31	90	95	34	36
F	4.48	94	85	32	38

million in females and from three to four and a half million in males, may be considered as representing about the same degree of anemia. Nine cases occur in this group. In 6 the mean corpuscular volume was 100 c μ or higher and therefore strikingly greater than normal. In 2 cases it was 98 c μ and still distinctly above normal and in only 1 case was the mean volume at the upper limit of normal values. Again, even when the anemia was very slight (red cell count over four million in females and over four and a half million in males) the mean corpuscular volume was 94 c μ or greater in 5 of 10 cases. It should be borne in mind that the values recorded represent only the first determinations in the cases cited, and were carried out for the purpose of differential diagnosis. In most instances corpuscular volume determinations were frequently repeated and all these subsequent observations showed results consistent with the statements above made. Fig. 1 illustrates the consistently high corpuscular volume in 3 of these cases observed over a period of three and a half months.

It must of course be borne in mind that the finding of a mean corpuscular volume greater than normal does not in itself justify a diagnosis of pernicious anemia. Such a finding simply classes the anemia as being of the macrocytic type. This form of anemia is also characteristic of sprue. Macrocytic anemia is frequently stated and rarely found to occur in association with carcinoma especially of the gastrointestinal tract, pregnancy and syphilis and is often associated with *Dibothriocephalus* infestation. I have also encountered several instances of macrocytic anemia in association with certain cases of diarrhea of obscure etiology, disease of the liver, aplastic anemia and anemia of acute blood loss. Such findings however are unusual.

In response to treatment with liver and the pernicious anemia liver extracts, more and more red corpuscles of normal size appear and eventually when

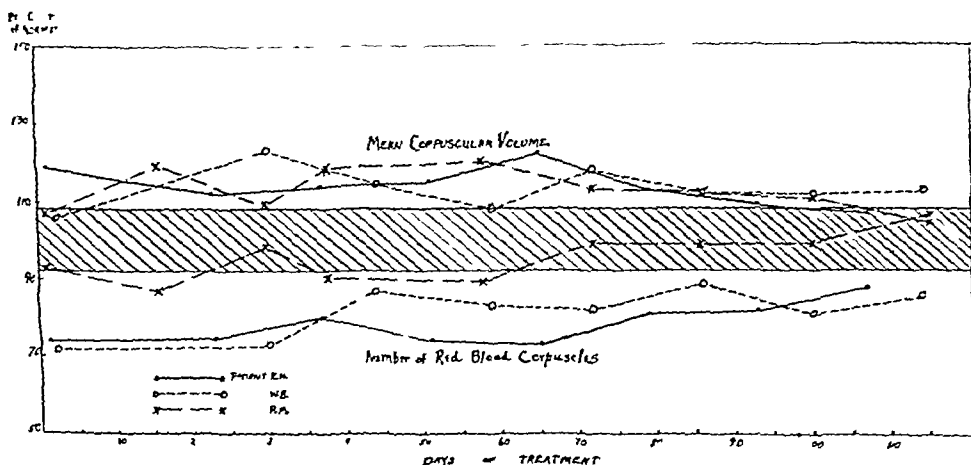


Fig. 1—Three cases of pernicious anemia observed over a course of three and a half months demonstrating increased mean corpuscular volume in spite of slight degree of anemia. Both mean corpuscular volume and red cell count are represented in proportion to the normal. The shaded area represents the range of normal.

the blood count has reached normal, normal or high normal values are encountered for mean corpuscular volume. This is true for sprue as well as for pernicious anemia."

Alterations in mean corpuscular hemoglobin are similar to those described for mean corpuscular volume. They may however be of less magnitude. For this reason determination of mean corpuscular hemoglobin may be of less value than the calculation of mean corpuscular volume. Furthermore volume determinations are less open to error since the accuracy of the hematocrit is much greater than that of the majority of hemoglobin methods.

Hypochromic Microcytic Anemias—In most types of anemia mean corpuscular hemoglobin concentration remains normal or at least little reduced. This is as much true of the simple microcytic forms associated with chronic toxic and infectious diseases as of the macrocytic anemias. There is a group of anemias however in which mean corpuscular hemoglobin concentration is significantly reduced. This group is also characterized by microcytosis and I have therefore suggested the term hypochromic microcytic anemia to dis-

tinguish this group from the anemias in which there is microcytosis without hypochromia (simple microcytic anemia) ²⁸

It is characteristic of this group to find a low mean corpuscular volume (70 to 55 c μ) Mean corpuscular hemoglobin is, however, more than proportionately reduced (20 to 14 $\gamma\gamma$) so that the mean corpuscular hemoglobin concentration is distinctly low (29 to 21 per cent) This is in striking contrast to the findings in other types of anemia (Table V) Measurement of the diameter of

TABLE V
SIZE AND HEMOGLOBIN CONTENT OF ERYTHROCYTES IN VARIOUS TYPES OF ANEMIAS

TYPE OF ANEMIA	MEAN CORP VOLUME c μ	MEAN CORP HBG $\gamma\gamma$	MEAN CORP HBG CONCENTRATION %	MEAN CELL DIAMETER μ
Microcytic	95 160	30 52	31 38	7 5 9 6
Normocytic	80 94	27 32	33 38	6 7 8 0
Simple Microcytic	72 79	22 26	31 38	6 5 8 5
Hypochromic Microcytic	50 71	14-21	21 29	5 8 7 5

the erythrocytes is of less value for, although the Price-Jones curve shows a broadened base, the mean diameter is usually little reduced and may even be within normal limits ^{15, 23}

Microcytosis with hypochromia commonly occurs as the results of chronic blood loss and is characteristic of the achlorhydric anemia described by Faber,¹¹ Witts,⁴² Kaznelson,²⁰ and others ²³ Hypochromic microcytic anemia is not always associated with achlorhydria, however but is common in persons who have partaken of a diet defective in hemoglobin building substances over a long period of time This type of anemia probably occurs whenever there is a lack of hemoglobiniferous substances in the diet, when these substances are improperly absorbed or utilized, or when there is excessive drain on the hemoglobin content of the body as in chronic blood loss

It is in this type of anemia that large doses of iron are so spectacularly effective Under such treatment more and more cells of normal size and hemoglobin content appear, the mean corpuscular volume and mean corpuscular hemoglobin concentration gradually rise, and finally normal values are attained Pernicious anemia liver extract is valueless in these cases and even whole liver seems to have little effect Here again, then the determination of the size and hemoglobin content of the red corpuscles is of value, for it not only facilitates diagnosis but affords information which is of great value in indicating the appropriate type of therapy

CONCLUSIONS

1 The differentiation of pernicious and other forms of macrocytic anemia and of anemias characterized by microcytosis and hypochromia from other forms of anemia is not only of academic and diagnostic interest but is essential in the guidance of appropriate treatment

2 Such differentiation is best made, from the standpoint of simplicity and accuracy, by the calculation of the volume and hemoglobin content of the red

corpuscles These calculations when combined with the simple examination of the blood smear yield more information at the expense of much less effort than is entailed in the measurement of the diameter of the red corpuscles

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THE SICKLE CELL PHENOMENON²

I THE RATE OF SICKLING IN MOIST PREPARATIONS

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ATTENTION was first directed to the phenomenon of sickling in 1910 when Herick¹ reported the presence of "peculiar elongated and sickle-shaped red blood corpuscles" in both fixed and moist preparations from a case of severe anemia. Emmel³ in 1917 observed that there was an increase in the number of sickled cells when the blood from a case of sickle cell anemia was left standing in sealed coverslip preparations. He also demonstrated the delayed appearance of sickled erythrocytes in the blood of the nonanemic father of the active case. Huck⁴ in 1924 noted that the sickled cells assumed a spherical form in from three days to six weeks. Since that time numerous experimental observations have been made on the sickle cell phenomenon most of which have been based on the behavior of red cells in whole blood or in various solutions when sealed under a coverslip. A review of the now rather extensive literature reveals that many figures have been given for the percentage of sickled cells appearing in a given case in a given time but no systematic study has been reported as to the rate of sickling over the entire course of the process. There has been disagreement as to the comparative speed of sickling in sickle cell anemia cases and in cases showing the capacity for sickling under suitable conditions but without the characteristic hemolytic anemia. The reliability of moist preparations as a method for further experimental work has yet to be established.

In this article the observations and discussion will be limited to the rate of sickling in moist preparations to a comparison of the rates in different clinical groups and to an analysis of some of the sources of error in the method. The reader is referred to recent comprehensive reviews of the literature by Graham and McCarty,⁵ Steinberg,⁶ and Yater and Mollari⁷ for a discussion of other phases of the problem and for a complete bibliography.

METHOD

Moist preparations were made by taking a drop of blood from a finger puncture on a new alcohol cleaned No. 1 coverslip, inverting on a new alcohol cleaned slide and allowing the drop to spread without pressure except for the weight of the cover glass. The edges of the coverslip were rimmed with vaseline using a small brush. The preparations were examined immediately at two- to four-hour intervals during the first twelve hours and at longer intervals thereafter. The number of readings are indicated by the points on the charts. The preparations were all kept at room temperature. Those which showed evidences of drying at the edge of the drop were interpreted as having insecure seals and were discarded. Preparations with air bubbles trapped within the drop were not used.

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No attempt was made to record the percentage of sickling after hemolysis became evident or in slides in which the cells were fragmented. The percentage of sickled cells was determined for each reading by counting one hundred cells, using the high power objective, and noting the number of cells in this group having definite, pointed projections. The personal equation in the interpretation of what constituted a sickle cell was the same throughout the study, for the readings were all made by the writer with the exception of an occasional count done under direct supervision. Where sickling was not uniform throughout the preparation, the percentage was determined on the basis of an average between the most sickled part of the field and the least sickled. In order to compensate somewhat for the variations in preparations from the same patient made under supposedly identical conditions, most of the readings were made on three or more most preparations, and the average of these curves taken as representative of the individual.

In the selection of cases to represent the types designated as sickle cell



Fig 1—Microphotograph of a moist preparation from a case of sickle cell anemia showing the characteristic bizarre and pointed forms

anemia and sickleemia, the following criteria were used. The sickle cell anemia patients were all negroes having a marked anemia, characterized by definite blood destruction manifestations (jaundice, increased icteric index, urobilinuria) and an associated regenerative picture (immature erythrocytes, increased reticulocyte counts, leucocytosis, thrombocytosis, etc.). They all gave a history of previous attacks of ill health, of joint and abdominal pains, and of leg ulcers. At the time the studies were made, they were patients in the hospital with the classical anemia and febrile symptoms associated with the active phase of the disease.

The sickleemia patients were taken at random from a series of cases found to have the sickle cell trait when a survey was done to detect this anomaly. (This survey now includes the examination of 827 negroes, with an incidence of 8.2 per cent.) These patients had no history, signs, or blood picture suggestive of a hemolytic anemia. Although complete blood studies were not done on the cases in this group, stained smears and reticulocyte counts revealed no evidence of abnormal erythropoiesis.

One patient, a borderline case who on admission showed the blood and clinical picture of sickle cell anemia but who later presented no evidence of an active hemolytic process does not fit either group, and the curves for this case are given separately.

OBSERVATIONS AND DISCUSSION

The rate of sickling in 13 cases of sickle anemia is given in Fig 2. In two of these patients the curve is taken from only one preparation, but in the others, three or more preparations are used in arriving at the composite curve for each individual. In Fig 3 the rate of sickling in five cases of sickle cell anemia is represented. In each series the total number of preparations examined was 39. The average rates of sickling from these two groups are compared in Fig 4.

SICKLING IN MOIST PREPARATIONS FROM SICKLE ANEMIA PATIENTS

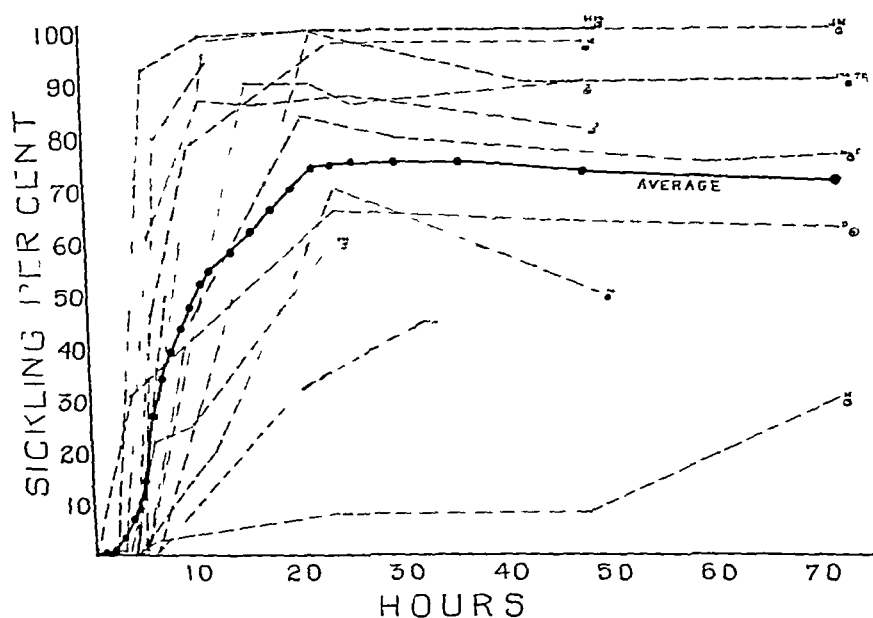


Fig 2—The rate of sickling in moist preparations from 13 cases of sickle anemia

From these observations it is evident that in our series sickled erythrocytes are often observed in moist preparations from sickle cell anemia patients as soon as the preparations are made but are never present immediately in sickle anemia patients. Moreover the rise in sickling occurs sooner and the total number of erythrocytes sickled at any given period during the first three days is greater. Although the return to the spherical form was not followed over a long enough period to compare adequately the speed of the rounding-up process in the two series the tendency is for the percentage of sickled cells in both groups to decrease after twenty-four to thirty hours with a sharper fall in the sickle cell anemia group.

These findings are in agreement with those reported by Huck² who noted in patients with severe, mild, and with no symptoms that the sickling percentage

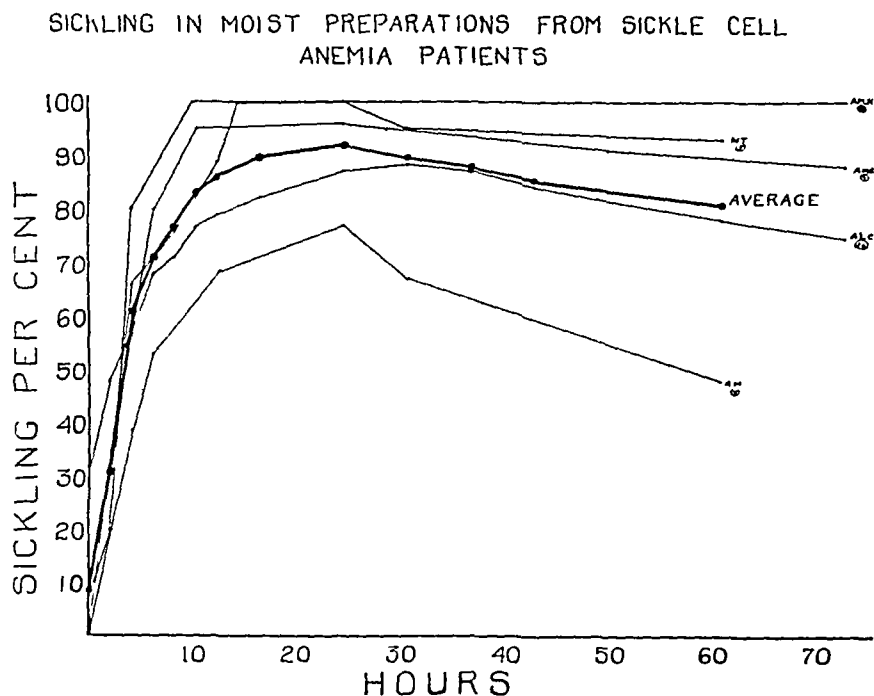


Fig 3—The rate of sickling in moist preparations from 5 cases of sickle cell anemia

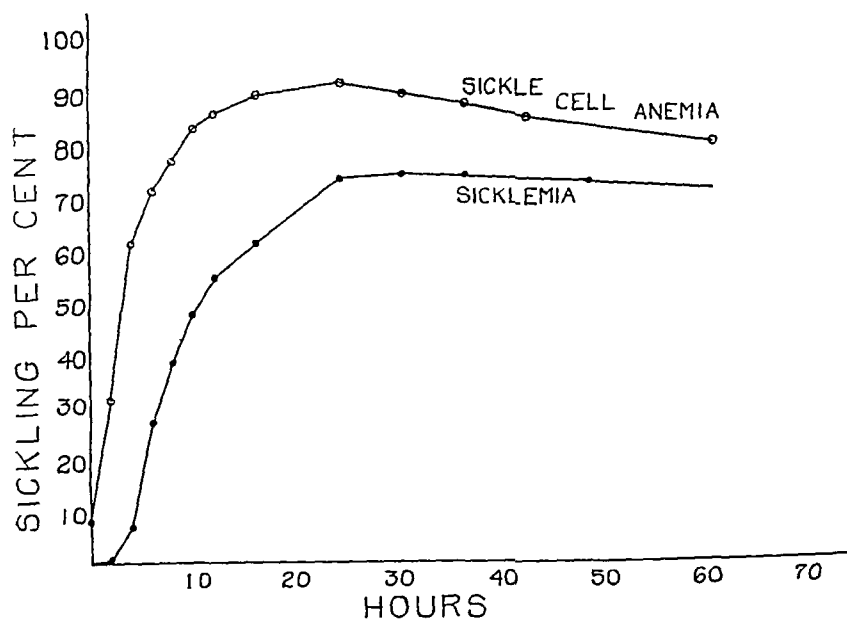


Fig 4—A comparison of the average rates of sickling in moist preparations in sicklelema and in sickle cell anemia cases

was almost 100 per cent 75 per cent and 25 per cent respectively. Other workers have noted the same differences in anemic and nonanemic cases.² The commonly observed fact that sickled cells are often found in smears from sickle cell anemia patients and are not present in sickle cell patients is also in agreement with these findings.

Other observers however have failed to correlate the speed of sickling with the clinical state. Cooley and Lee⁵ did not find that sickled cells occur more readily or in greater proportion in the blood of a sickle cell anemic child with a mild grade of hemolytic jaundice than in other children without symptoms. Stewart⁶ stated that there is no relation between the severity of the clinical picture and the percentage of sickled cells present. Hahn¹⁰ noted that although

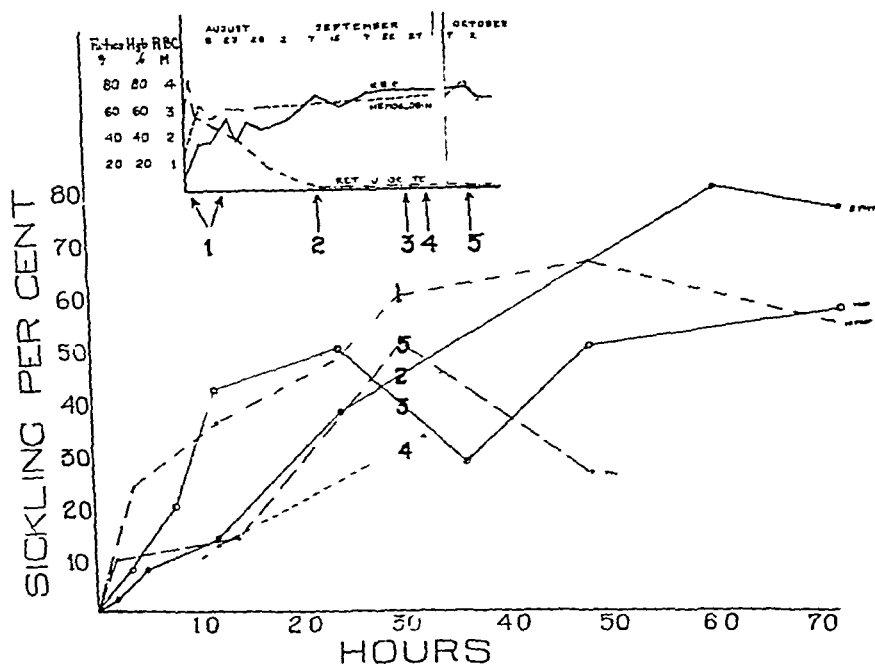


Fig. 5.—The rate of sickling in moist preparations from a mild case of sickle cell anemia made at various times during a period of improvement.

sickle cell anemia patients have sickled cells in their circulating blood "there is no difference in the ability of the red corpuscles of anemic and nonanemic subjects to form sickle cells outside of the body under suitable conditions."

In the interpretation of these seemingly contrary observations certain factors must be taken into consideration. The frequent finding of 100 per cent sickled cells after a sufficient interval of time in individual preparations from sickle cell patients as well as from cases of sickle cell anemia indicates that the capacity of the cells to sickle in the two clinical types under suitable conditions may be the same. In the moist preparation however it is not a question of how many cells could sickle under ideal sickling conditions but what percentage of the cells assume bizarre forms under the conditions of the moist preparation. Also the variability of individual preparations and conclusions drawn from a few

observations may lead to false ideas as to the average reaction. Another, and probably a very important factor is that patients with sickle cell anemia vary considerably in their speed of sickling, and that mild cases or those with a temporary anemic episode of a hemolytic type may give the sickling characteristics of the inactive group. Conclusions drawn from such a case would not hold for the more chronic cases.

The rate of sickling in such a mild case of sickle cell anemia is represented in Fig 5. The patient, a negro girl of three, was admitted with an erythroblastic type of anemia, with numerous nucleated red blood cells, a high reticulocyte and leucocyte count, and with an increased icteric index, etc. There were no typical sickled cells in the fixed smear, and the sickled cells in the moist preparations appeared slowly and in low percentages. The patient improved clinically, her hemoglobin and red blood cell count rapidly increased, the reticulocyte count dropped, and the immature erythrocytes disappeared from the blood picture. Series of moist preparations made during this return to the sickle type of condition showed no significant changes in the sickling rate. Although there persisted a low grade of anemia of the defective formation type, the patient after returning home showed no more clinical or actual evidence of anemia than a brother and a sister who likewise possessed the sickle cell trait, or another brother whose cells did not sickle in moist preparations. (The studies on this case during the active phase, were made during the extremely hot summer months when vaseline was a liquid and paraffin was necessary as a seal. This may have influenced the results, but we have noted no difference in the use of these two kinds of seals in other cases.)

Although the evidence is as yet limited, we are definitely convinced from our experience with a fairly large group of sickle cell anemia cases that the rate of sickling is related, not necessarily to the immediate severity of the anemia, but to the chronicity of the anemia. Those with no sickled cells in their circulating blood, with a slow rise in percentage and with a low maximum percentage have a better prognosis than the "high-immediate, rapid-rise, high-percentage" group.

The variability of moist preparations and the unreliability of a single moist preparation as representative of the average sickling rate for a given blood, as emphasized by Graham⁴ and noted by other investigators, has been amply verified in our studies. Preparations quite often show variations in percentage in different parts of the same microscopic field. Sickling less than 1 per cent may be seen in one preparation while another made from the same patient at the same time under similar conditions may show 100 per cent. Sickle cell patients are more likely to show a more marked variability than are sickle cell anemia cases. In 194 preparations from cases known to possess the sickle cell trait, in which the rate of sickling was followed at frequent intervals for three days, 5 showed no cell changes. Although this is an error of less than 3 per cent, the fact that such a thing is possible is worthy of note. Preparations have been observed which showed no sickling in twenty-four hours, but became positive in fairly high percentages in forty-eight or seventy-two hours or longer. The difficulties in distinguishing sickled cells from other morphologic changes in erythrocytes, such as marked crenation, dying and fragmentation phenomena, poikilocytosis, elliptical cells and changes produced by pressure, may, at times,

be great. The present methods do not take into consideration such factors as method of collection, exposure to air, size of drop, character of spread, weight of the cover glass, hydrogen ion concentration of the blood after placement in the sealed preparation, bacterial growth, minor variations in temperature, exposure to light, and effectiveness of the seal. The fact that we cannot always produce the same curve in the same person is evidence of the presence of as yet uncontrolled factors and clearly indicates that we will have to consider these sources of error in the interpretation of results and in the incidence of sickling as at present reported.

The use of the gas chamber method, as advocated by Hahn and Gillespie¹¹ as a means of experimental study has in their hands led to the most important fundamental observations concerning the sickle cell phenomenon, but in our hands it has been cumbersome and unreliable. Weeks spent in attempting to develop the technique to a point where it would be usable as a practical measure for conducting a survey met with failure, although the feeble responses we obtained were in agreement with the findings reported by these workers. Graham,⁴ Cooley,¹² and Scriven¹² have experienced the same difficulty. Others have reported positive results with the gas chamber method, but have not repeated the more detailed observations.

At the present time, therefore, we lack reliable methods. New procedures or a better control of the variable factors in the present methods are necessary before the interesting sickle cell anemia problem can be unravelled.

CONCLUSIONS

1. The erythrocytes in whole blood in moist preparations from patients with sickle cell anemia sickle faster and in a higher percentage than in sickle cell patients.

2. Twenty-four hours is the optimum time for the reading of moist preparations, but negative readings at twenty-four hours do not eliminate the possibility of the sickle cell trait.

3. The rate of sickling is extremely variable and conclusions that will be representative of the group or of the individual cannot be drawn from single cases or from a few preparations.

Appreciation is expressed for the skilled technical assistance given in this work by Miss Juanita Bibb, and for the cooperation of the clinical staff of the Memphis General Hospital.

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CLINICAL APPLICATIONS OF SUPRAVITAL STAINING*

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A COMPREHENSIVE definition of supravital staining is difficult. It implies, in general, the staining of living tissues independent of the organism as a whole. Specifically, it is used to indicate the staining of living cells which have been removed from the organism, or which are stained after the somatic death of the animal.

The physical and chemical laws which govern the suitability of substances for supravital staining are understood only in part. Cappell² gives an excellent definition of the practical working basis upon which this may be determined. He states that "the latter," i.e., supravital methods of study "require agents which, while not toxic to the cells, are yet capable of producing their maximum effect within a few minutes," etc. The fundamental investigations of Schultze²⁶ concerning the chemical and physical characteristics of vital dyes established the fact that a substance must belong in the borderland between colloids and crystalloids in order to be an efficient vital stain. This fact holds relatively true for supravital dyes. General laws concerning any other factors which regulate the suitability of a substance for supravital staining have not been determined. So far the best supravital stains have proved to be weak basic dyes.

Simpson²⁷ made an extensive study of the suitability of a large number of both acid and basic dyes as supravital stains for blood. She took into consideration the toxicity of the dyes, the length of time necessary for satisfactory staining, the permanency of the color once staining had been obtained, the specificity of the staining for specific granules and for mitochondria, and the suitability of the dyes for revealing the vacuolar apparatus. She found that neutral red fulfilled the requirements of a satisfactory supravital dye for specific granules and phagocytic vacuoles better than any of the other dyes tested, and that janus green B was one of the best stains for mitochondria. Furthermore she found that these two dyes in combination were especially satisfactory for depicting contrasts between mitochondria and vacuoles in blood cells. Sabin²⁴ also found these two dyes wholly satisfactory for the supravital staining of blood, and more satisfactory than the other dyes which she used. Utilization of these two dyes by other investigators in the field of supravital staining has yielded equally satisfactory results so that at the present time, these two stains, either alone or in combination have come to be the chief dyes commonly used in the supravital study of the blood. They are the only dyes which will be considered in the discussion which follows.

The technique of preparing the slides and solutions and of making the supravital smears has been given in detail elsewhere²⁸ and need not be repeated here. In brief the method consists of flooding clean glass slides with dilute alcoholic

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solutions of either neutral red alone, or of a combination of neutral red and janus green. The slides are at once drained and dried, and are then ready for use. A drop of fresh blood is collected on a coverslip, which is immediately placed on one of these stained slides. The blood must be allowed to spread by capillary attraction and without the aid of pressure. The edges of the preparation are rimmed with vaseline to prevent evaporation, and the smear is kept in a hot box at body temperature long enough to permit staining. Five or ten minutes are sufficient for the staining of the mitochondria. About twenty minutes are required for satisfactory staining of the vacuoles and granules. Janus green is quite toxic, and it is therefore necessary that blood stained with it be studied soon after preparation. It also inhibits, to some extent the staining with neutral red, so that an accurate determination of the size and number of the vacuoles must be made with neutral red alone. Furthermore, any estimation of the vacuolar activity must take into consideration the length of time that the blood has stood in contact with the stain, as the vacuoles increase in number, size and PH reaction with time. We have published elsewhere^{8, 9} the exact requirements for a satisfactory supravital preparation as well as descriptions of the appearance of the normal white blood cells when freshly stained, and after having stood for various periods of time. These reports contain very accurate illustrations in color of the cells stained both with neutral red alone and with neutral red in combination with janus green. It seems wiser to refer any one wishing to study exact descriptions of supravital stained cells to those articles than to attempt at this place a repetition which, to be of any value, would have to be lengthy.

It can be seen from the foregoing brief review of the requirements for satisfactory supravital staining that there are some inherent disadvantages in the method. At first thought, these may make the method seem impracticable for any very general use, especially as some of the disadvantages do not occur in other methods of studying blood.

The chief disadvantage in the method is, perhaps, the fact that the smears cannot be kept long and therefore must be studied soon after they are made and cannot be saved for future reference or for shipment. The necessity of studying them within a given period of time sets an inflexibility that often may be difficult to meet. This is especially true if the preparations must be transported to any distance. Furthermore, transportation under any condition is somewhat difficult, as the slides must be kept flat to prevent flowing of the cells to one side of the preparation. In fact, in most cases, the method does not lend itself to instances where the blood is to be examined at any great distance from the place where the preparations are made.

The need for a hot box or some other method of keeping the blood at body temperature may also at times cause embarrassment. Where the microscope is kept in any permanent place and the blood brought to it, this offers no problem, as a permanent heating system can easily be set up. It is only in instances where the microscope is carried to the blood that there may be difficulty. This difficulty can be met by the use of a portable hot stage.

Preparation and staining of the smears are without any doubt simpler and less subject to error than is the case with fixed smears, but the preliminary clean-

ing of the glassware is somewhat time-consuming. For properly made fixed smears to be sure the coverslips need as careful cleaning as for supravital preparations. But in the latter case, the slides must also be subjected to the same careful attention. Since it is as easy to clean and stain a larger number of slides as a few, the time required for their preparation can be materially minimized by the preparation of a large supply. They can be stored indefinitely if kept away from dust and grease.

More time and skill are necessary in learning to use the supravital method to its full advantage than is the case with methods of fixed staining. This is simply because the method depicts more cytologic factors and permits of the detection of more modifications in those factors than do other methods. For simple differential counting the method may be learned as quickly as any other, but for detection of all the possible pathologic modifications that may be observed in the various factors that stain supravitaly and for their interpretation considerable time and experience are necessary. It is much like the fact that it does not take long to learn the name and appearance of an intricate machine, but it requires considerable experience to learn all of the uses to which it may be put and all of the factors that may go awry with it. The supravital method of staining blood offers a tremendous range of factors for study.

There is one fact, however, which may offer difficulty for even routine differential counting. This is the fact that nuclei do not stain and therefore do not aid in the rapid detection of the nucleated cells within the field of vision. This is of no significance in the detection of the granulocytes and monocytes, since their granules and vacuoles, respectively, make them conspicuous. It does, however, make detection of lymphocytes and nucleated red cells more difficult than with fixed staining, where the nuclei are prominent. In the case of the red cells this is true with both the single and double staining. In the case of the lymphocytes it is true only with the single stain since mitochondria aid in the detection of lymphocytes with the double staining. To a trained observer the lack of nuclear staining is not a source of error, as both lymphocytes and nucleated red cells are easily visible in a supravital field when the source of illumination is properly adjusted and the smears are thin enough. Moreover, in most instances especially if the preparation has stood twenty or more minutes, the lymphocytes contain a few tiny vacuoles of neutral red which serve to bring them to the observer's attention.

Another objection to the use of the supravital technic in the study of blood is the fact that basophilia and stippling cannot be detected as such. Kervin has shown that the material, which can be demonstrated in young red cells by the appropriate use of certain supravital stains is the same substance which appears as stippling and basophilia in fixed smears. Reticulation may appear in some of the red cells in a supravital preparation after they have stood in contact with the stain for a considerable period of time, but for any accurate estimation of reticulation a concentration of neutral red must be employed which is too great to be compatible with the life of the cell and therefore with supravital staining.

Parasitic inclusions (in particular malarial parasites) also do not stain in supravital preparations until after a long exposure to the dye and therefore

may easily be overlooked at the time of the differential count. When they do finally take the dye, they are very prominent.

It will be seen from the foregoing list of the disadvantages of the supravital technic that the principal ones are the impermanency of the preparations, the necessity of examining them soon after they are made, the impracticability of transporting them, and the difficulty of detecting parasitic inclusions and certain pathologic features in circulating red cells, i. e., nuclei, stippling, basophilia.

In a consideration of the use of any particular technic, it seems reasonable to consider its advantages and the information obtained from its use, as well as its disadvantages, and to contrast these with the advantages, disadvantages and information inherent in other related technics. In the case of the supravital technic for the study of blood, a number of cytologic characteristics can be demonstrated which cannot be seen in fixed preparations. Furthermore, some of the characteristics which can be seen in fixed preparations appear in a somewhat different manner in fresh preparations. It is necessary to know wherein the cytologic features presented by the two methods vary, and in what clinical conditions any particular set of features is of importance before one can draw any conclusions as to the technic of choice for the study of blood in general, or in any particular instance.

In supravital preparations the cells are living and may be considered more or less in the physiologic state in which they occurred in the body at the time that they were withdrawn. This statement, of course, holds true the sooner the preparation is examined after it has been made. For that reason it is considered important to study the cells as soon as they have taken sufficient stain to bring out their cytologic features. As has been mentioned elsewhere, this is from twenty to thirty minutes after preparation of the smear. It is obvious that a study of any physiologic characteristics that can be made observable might be of diagnostic and prognostic aid wherever pathologic states of the host cause modifications in those characteristics, or where the cells are inherently pathologic in themselves.

The important physiologic factors which are demonstrated by the supravital technic, and are not demonstrated in fixed preparations, consist of motility, mitochondria and the vacuolar apparatus. The motility varies in rate and character in the different types of cells, and therefore can be used to help in their differentiation. The rate of motility in all of the cells is subject to considerable variation due to trauma in making the smears, to the temperature at which the smears are kept, to the age of the cell, to pathologic conditions of the cell, and finally to factors in the environment which are not understood. Obviously, where any activity may be affected by so many factors, and where those factors are only partly controllable, decisions based upon that activity can be made only with reservation. In the case of cellular motility, therefore, the increased or decreased activity, of any strain of cells, can be judged only in relation to the activity of other cellular types in the same smear. So far as has been reported, there is no evidence that the form of motion in any given type of cell changes. It is merely the rate of activity which seems to be modifiable. In the differentiation of cellular types, the absence of motility is of very little signifi-

cance, as cells may remain quiescent for long periods. The presence of motion, on the other hand, may be of considerable assistance. This is especially true in the case of the mononuclear cells. Cunningham, Sabin and Doan⁷ found that both monocytes and lymphocytes were motile, while on the other hand, they never observed movement of myeloblasts or of myelocytes until the cells had practically become adult polymorphonuclear cells.

The staining of the vacuolar apparatus in blood cells is perhaps the most valuable feature demonstrated by the supravital technique. The aid offered by the vacuolar apparatus in the differentiation of mononuclear white blood cells has received considerable attention, but the importance of the modifications within the vacuolar apparatus under various pathologic conditions is just beginning to receive recognition and to be considered of diagnostic or prognostic help. The staining of the vacuoles in monocytes is particularly prominent, and particularly subject to modifications. Simpson and Sabin showed that the vacuolar apparatus in the monocytes is very distinctive and serves as an accurate differentiation of monocytes from lymphocytes or from young mononuclear cells of other series. They stressed the number of vacuoles and the ground glass appearance of the cytoplasm in the monocyte as offering a marked contrast to the few vacuoles and the clear hyaline-like cytoplasm of the lymphocytes. To these major features in the characterization of the monocyte Sabin added the size and arrangement of the mitochondria, the quality of the unstained nuclei, and the tendency to rosette arrangement of the vacuoles. Cunningham, Sabin and Doan, and Cunningham and Tompkins^{8, 9} agreed in the main with their descriptions, but showed that there is considerable pliability in the vacuolar arrangement dependent on whether the cell is rounded up or in motion. All of these authors found a much more extensive vacuolar apparatus in the monocytes (i. e., both more and larger vacuoles) than in the lymphocytes. While there has been no disagreement with the descriptions of monocytes given by the above authors, various reports show that the individual characteristics of the monocyte are not specific to that cell alone but may be observed in other mononuclear cells of the blood when supravitaly stained and cause confusion in their differentiation. The possibility of confusion between monocytes and lymphocytes has been particularly stressed. Bloom² studied the mononuclear cells in the blood and tissues of rabbits following experimental irritations. He found many cells which exhibited various stages of vacuolar activity and in which differentiation between lymphocytes and monocytes was impossible. Lawrence and Todd¹⁰ studied the blood in various clinical conditions in which the lymphocytes were admittedly involved and found many lymphocytes which had such an overactive vacuolar apparatus that they could not be differentiated from monocytes on the basis of the vacuolar apparatus alone. Hall¹¹ published a very careful and critical review of the hematologic literature concerning the results of supravital staining. In particular he examined the evidence dealing with the value of the supravital technique in the differentiation of monocytes. He concludes that the method does not help in the differentiation. Among other factors which led him to this conclusion was the extreme modifiability of the vacuoles and the ease with which their character is changed by pathologic and

environmental conditions. As reports of supravital studies of pathologic cases appear, the extent to which the vacuolar apparatus may be modified becomes more evident. At the same time, the probability arises that this liability of the apparatus may be of great value in differential diagnosis. Sabin showed that the vacuoles of the monocytes in Malta fever varied considerably. Wilson and Cunningham,³⁰ Rucks and Cunningham³¹ and Lawrence, Josey, and Young¹⁷ found all stages of development of the vacuolar apparatus in cases of monocytic leucemia. Cunningham and Tompkins,⁷ Blackfan and Diamond¹ and Rogers²² reported specific changes in the vacuolar apparatus of the monocytes in tuberculosis. Comparable variations have been observed in the vacuolar apparatus of lymphocytes under various pathologic conditions.³⁰⁻³⁸ Tompkins and Cunningham²⁹ have reported a variety of conditions in which they found modifications in the vacuolar apparatus of either monocytes, lymphocytes or granulocytes. In all of these reports, the modifications of the apparatus were regarded as specific to the pathologic conditions in which they occurred, and while they rarely offered any confusion in the differentiation of the cells, they were found to be of assistance in diagnosis.

The third factor of major importance demonstrated by the supravital technique in the study of blood cells is the staining of the mitochondria. Mitochondria, of course, have been demonstrated by methods of fixed staining, but these have not permitted of the simultaneous study of a sufficient number of other cytologic features. The simultaneous staining of the mitochondria and vacuoles in supravital preparations has a value that studies of the mitochondria alone in fixed preparations do not offer. It is obvious, in the first place, that simultaneous observation of a number of factors within a cell is preferable to the necessity of separate observations in different cells of the same type. Furthermore, it is obvious that there is less distortion of the intracellular inclusions in stained living cells than is likely after even the most felicitous fixation. As the size, shape, and arrangement of the mitochondria have been considered as important as their number in cellular differentiation, this feature assumes considerable value. Even under the conditions of supravital study of the mitochondria by the use of janus green, there has been found to be considerable variation in their number and form from cell to cell of a given type. Cowdry⁴ observed that mitochondria are extremely sensitive to physiologic and pathologic conditions. Despite their normal variations, however, there has been found sufficient similarity in the mitochondria in any one type of cell and sufficient difference in the mitochondria between types for a differentiation of blood cells to be made which is based partly on the mitochondrial pattern. This is especially true when neutral red is used in conjunction with janus green. By the use of these two dyes, together, Simpson and Deming²⁸ and Sabin, Austrian, Cunningham and Doan²⁹ were able to trace the development of granulocytes from myeloblasts through successive stages to the mature polymorphonuclear cells, and to define the myeloblasts and young myelocytes with a certainty not previously obtained. With the same technique, Cunningham, Sabin and Doan followed the development of leucocytes, lymphocytes, and monocytes from their stem cell. They discovered very useful and consistent differences in the cytologic

patterns of the young cells of each series which have made it possible to distinguish them from each other and from mature myelocytes, lymphocytes and monocytes. As in the case of the vacuolar apparatus, the variations which may occur in mitochondria due to both physiologic and pathologic causes may be of considerable diagnostic and prognostic assistance rather than a source of confusion. Cowdry realized this and discussed the probable importance of the sensitivity of mitochondria to pathologic changes. Taken alone, without consideration of the other cytologic factors stained by neutral red, the character of the mitochondria in the different cells and their modifications might well cause confusion. Studied in combination with other factors, the mitochondria are invaluable. In fact there are a considerable number of conditions in which the mitochondria have been found to be specifically characteristic.^{20 18 29}

From the foregoing remarks it is obvious that supravital staining permits of observations of motility, vacuolar apparatus and mitochondria and that these three factors are subject to modifications under various environmental, physiologic and pathologic states. Despite the modifications, the pattern of these three factors in each type of cell seems sufficiently characteristic to be of distinct value in the differentiation of these cells. The aid obtained in cellular differentiation is of especial value in the case of the young mononuclear cells. On the other hand, the modifications in the mitochondria and vacuolar apparatus are sufficiently specific to the conditions which elicit them for them to be of aid in the differential diagnosis.

In addition to these three major factors brought out by the supravital technique, there are various other factors which may be observed with other techniques, but which at times may be more accurately measured in the living cells. These include measurements of the diameter of cells, abnormalities in the shape of cells, the color of red cells, and the presence of any cytoplasmic inclusions, such as fat, which might be destroyed with fixation. The distortions in the shape of cells which often occur in pulled smears are avoided in living preparations where the cells are free to assume their natural shapes. This feature has its greatest importance in the detection of sickle shaped red cells, which Lawrence¹⁷ found to be more reliably distinguished in fresh than in fixed preparations. The production of smudges which often results from the trauma of pulling smears, especially in pathologic bloods with very fragile cells, is also avoided in supravital preparations. It is obvious that the elimination of smudges adds to the accuracy of the differential counts and of the detection of pathologic cells.

Estimations of the color of red cells can be made with extreme accuracy in supravital preparations in which basophilia and variations in the intensity of stain do not interfere with the color due to the hemoglobin alone. In addition it is easy to detect the faint traces of hemoglobin which occur in the very young red cells, and which are concealed by intense basophilic staining. The detection of hemoglobin simplifies the differentiation of very young red cells from young white cells.

Pomphkins and Cunningham found that fat occurs occasionally in the white blood cells under certain pathologic conditions and may aid in diagnosis or prognosis. They found that in extremely toxic infections and especially in

moribund states, the polymorphonuclear neutrophiles may contain highly refractive, unstained droplets which they consider to be fat. In two cases of generalized lymphosarcomatosis they found similar droplets in the lymphocytes. Cunningham, Sabin, Sugiyama and Kindwall⁶ found that fat was consistently present in degenerating epithelioid cells.

From this review of the important features connected with the supravital technique, one is in a position to analyze the conditions in which the specific information obtained from supravital staining is likely to be of help in diagnosis or prognosis. It has been shown that the method is of value first in the differentiation of mononuclear cells of various types, including the young forms of the different strains of white blood cells, and secondly, in showing qualitative differences in any single type of mononuclear cell. In this latter respect, the method is particularly helpful in depicting qualitative changes in the lymphocytes and monocytes. Therefore, the method is likely to give assistance in any condition involving the mononuclear cells. This is true, whether it is a condition involving the lymphocytes or monocytes, or a condition associated with the appearance of young cells.

In the case of diseases involving the lymphocytes, it has been found that the increases in those cells in certain diseases, such as pertussis,¹⁸ lymphocytic reactions to sepsis,^{30, 18} and tuberculosis,⁷ are due to cells that are normal in every respect, while other diseases are characterized by the appearance of lymphocytes which stain in the manner of young cells. The infections of childhood, typhoid fever, malaria, and measles²⁹ are the common conditions in which young lymphocytes may be found. On the other hand, the lymphocytes in infectious mononucleosis are different from either normal or young cells.^{30, 18, 29} The lymphocytes of lymphatic leucemia have been shown to exhibit characteristic changes in mitochondria, vacuolar apparatus and nuclei which help to differentiate them from other conditions involving the lymphocytes, as well as from other mononuclear cells.^{30, 18, 29}

Supravital staining has proved especially valuable in diseases involving the monocytes, because it has aided not only in the detection and differentiation of these cells, but has revealed sufficiently characteristic changes in them in a number of pathologic conditions for the modifications to be of value in diagnosis. Morriss and Tan,²⁰ Cunningham and Tompkins,²⁹ Blackfan and Diamond,¹ Rogers²² and Finner¹¹ have found the monocytic count and the relationship of monocytes to lymphocytes to be specifically involved in tuberculosis. Sabin found the monocytes numerically and qualitatively changed in Malta fever. Mulhgan²¹ and Tompkins and Cunningham found them affected in malaria. The latter authors found them stimulated in their capacity to store dye in a group of conditions with associated hepatic disturbances, and also as an accompaniment of pyogenic infections. They also found them depressed in their capacity to store dye in conditions associated with anemia. The diagnosis of a third type of leucemia, i. e., monocytic leucemia, has been made more certain by supravital blood studies.^{30, 17, 20} As a result of these studies it has been possible to accurately differentiate the condition from lymphatic and myelogenous leucemia.

In fact in the leucemias in general, supravital staining with the double stains has proved of tremendous value in the separation of the three main types, and in the classification of those types according to their chronicity. Detailed descriptions have been published of myeloblasts and myelocytes at various stages of maturity in the blood of cases of myelogenous leucemia, and the points have been discussed by which they may be differentiated from other mononuclear cells.^{25 25 29} It has been found that myeloblasts and very young myelocytes characterize the more acute stages of the disease, while late myelocytes are characteristic of the more chronic stages.^{25 29} It has also been found that an accurate estimation of the number of young myelocytes, compared to the number of late myelocytes, is of help in distinguishing severe reactions to pyogenic infections from aleucemic stages of myelogenous leucemia.²⁹

It is impossible to lay down dogmatic rules as to when any one method of studying blood should be employed. There are times, as every one knows, when a number of methods are necessary to give enough information for diagnosis. There are times when any satisfactory method will give all of the necessary information. In regard to the use of the supravital technic, it would seem as if its value in the analysis of mononuclear cells should, wherever possible, make the technic a routine in all diseases involving the lymphocytes and lymphoid tissues, in all questions of leucemia of any sort, and in all conditions in which the number or character of the monocytes have been shown to be of diagnostic aid. These latter conditions include tuberculosis, malaria, anemias and conditions accompanied by hepatic disturbances, such as arsphenamine toxemia, catarrhal jaundice, and Malta fever.

Besides these special cases when supravital studies seem routinely indicated, the method should also be employed, in addition to fixed staining, wherever fixed staining has given indications of abnormalities of the mononuclear cells, or of the presence of young white cells.

In the study of anemia, the method can also be profitably employed at times for accurate estimations of the color of the red cells as well as for determinations of the size and shape of the cells, and for detection of the very young red cells, the megaloblasts. It is especially indicated where there is any question of the presence of sickle cells.

In the course of acute infections the method is useful in differentiating the infectious conditions from aleucemic myelogenous or lymphatic leucemias. In addition, in infections, the method may give information concerning the toxicity of the infections, and concerning the activity and youth of the granulocytes, and therefore of the regenerative powers of the marrow.

With the exception of the above conditions, when supravital studies seem specifically indicated there is no reason to prefer them. Fixed preparations are of course necessary when permanent smears are desired.

In addition to the use of the supravital technic in the study of blood, the method offers some distinct advantages in the study both of body fluids, and of scrapings of various tissues. The possible applications of the method in these directions have but just begun to be investigated and very little has as yet been published concerning such studies. Cunningham and Tompkins³⁰ found that

supravital studies of tuberculous exudates showed great numbers of epithelioid cells and were of diagnostic importance. McJunkin¹⁰ made supravital studies of the lymphoid tissues in Hodgkin's disease. Forkner^{12, 13} made an extensive investigation of both normal lymph nodes and nodes from various pathologic conditions, including lymphosarcoma, Hodgkin's disease, lymphatic leucemia, and tuberculosis. He devised a very simple method for biopsy extraction of enough material from the nodes for supravital studies, and found sufficiently characteristic changes in the tissues under the different pathologic conditions to encourage further investigation and a restricted application of his methods to selected cases. Doan¹⁰ applied the method to the study of marrow from cases of pernicious anemia and obtained invaluable information concerning the phagocytic cells and the regeneration of the red cells.

The technic for such studies is essentially the same as that for blood. The strengths of the dyes which give the most satisfactory results vary according to the tissue to be studied. Injury to the cells must be avoided in making the preparations. Smears of fluids are made in the same manner as those of blood. Smears of tissue scrapings are also made in the same manner whenever they are moist enough in themselves to permit of spreading. Pieces of solid tissue must be carefully avoided and pressure is fatal to the cells to be studied. Where the tissues are not sufficiently moist to spread normally, the scrapings may be mixed with a drop of homologous serum and smears made from the mixture. Saline solutions are not suitable for this purpose, as they cause too great an edema of the cells, with a consequent distortion of the cytologic figures.

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HEMOPHILIA*

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HEMOPHILIA is a condition characterized by a delayed clotting time of the blood and a history of repeated hemorrhages, which is found only in males but is transmitted through the female as a sex-linked character. Thus, it will not appear in the children of a hemophilic man but may be transmitted by his daughters to appear in their sons. The disease will not appear in the sons of any male descendant of a hemophiliac (except in case of intermarriage between two such families) but may crop out at any time in the sons of the female descendants. This law of hereditary transmission of the disease was first promulgated by Nasse in 1820¹ and has since then received repeated verification, especially by Bulloch and Fildes² in their critical investigation of the literature of reported family histories. Gates³ has very recently given an excellent survey of the literature along this line which should be read by anyone particularly interested.

Each hemophilic male, therefore, who marries and has children serves as a new starting point of the disease in a hereditary sense. This is well illustrated by a family history which I recently presented.⁴ The drawing of this family tree is reproduced here to emphasize the law of transmission of the disease. Widespread diffusion of the disease in recessive form, which has taken place with the passage of centuries, no doubt accounts for the numerous cases seen in which no positive family history is obtainable. There is no way of being certain, however, that it does not occur spontaneously. Intermarriage between members of different or of the same hemophilic family usually proves disastrous, as the disease at once becomes rather dominant in character. This has happened in several of the royal families of Europe through their close intermarriage.

Etiology—Our knowledge of the origin of the disease is no further advanced than it was a century ago. Pickering⁵ found certain points of similarity between hemophilic blood and that of certain embryonic forms, and obtained beneficial results from liver feeding in two cases. He therefore postulated some kind of faulty liver development as a basis for the condition. Mailow⁶ has recently repeated Pickering's attempt at liver therapy in 4 cases, but with no success whatever. It is therefore unlikely that the liver is concerned in the disease, although it is intimately related to blood clotting factors in other ways.

Perhaps the most important work so far reported on the etiology of hemophilia is that of Birch.⁷ Working on the assumption that females of hemophilic families must possess some protective factor against the disease, which is lacking in the males, she tried ovarian transplants and ovarian extract therapy in hemophilic males. Apparently remarkable success has attended this type of therapy. She also went one step further, and tested the urine of hemophilic males for the

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small trace of female sex hormone which is usually found in male urine. In her five hemophilic cases no trace of this hormone was found. She, therefore, believes that the disease is kept in recessive form in the female carriers by the activity of their sexual organs, and that it crops out in certain male members of the family because of an entire lack of the small amount of female sex hormone normally present in males. Much work remains to be done before her proof is complete, for instance whether there are nonhemophilic males who excrete no female sex hormone in the urine.

Pathology—Most of our knowledge of the pathology of hemophilia is concerned with the coagulation of the blood. Attempts to show the presence of undue friability of the capillaries or alterations in the coagulative property of the tissue juices, have yielded no convincing results.

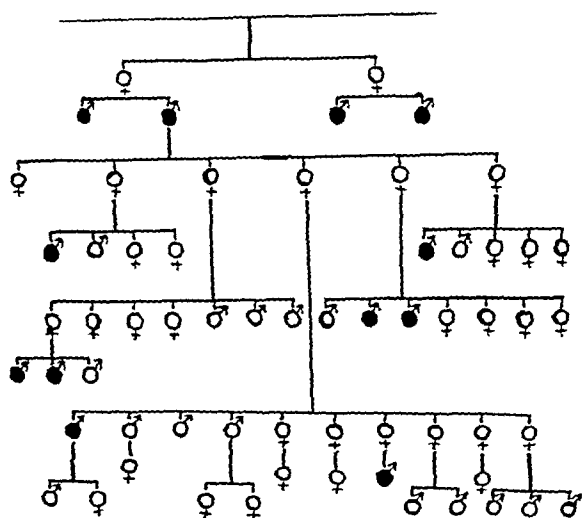


Fig 1—Genealogy of the Prickett family with bleeders shown in black. No earlier history was obtained.

The blood exhibits as its major abnormality a *prolonged clotting time*. This may vary anywhere from normal limits up to several hours, depending on the severity of the condition and the time at which the blood is taken. One person may show wide fluctuations of his clotting time at different periods of the year, at one time being apparently normal and a few weeks later going into a severe hemorrhagic state with greatly delayed clotting. This marked variability in the clotting time is characteristic of the disease and should always be kept in mind. The *bleeding time* from a stab wound in the finger or ear is usually normal^{9, 10} although at times it becomes greatly prolonged if the wound be treated a bit too roughly. The cellular elements of the blood are normal in character and amount except for the secondary anemia following severe bleeding. Recovery from this anemia is usually very prompt. The *blood platelets* are normal in number and size but exhibit a delayed clumping and disintegration which has been considered by some workers^{10, 11} as an important factor in the prolonged clotting time. There has been some disagreement^{10, 12} as to whether the platelet

material liberated by the disintegration, possesses the coagulative power that it normally should

Prothrombin of the blood in hemophilia seems to be the only clotting factor exhibiting definite abnormality. Howell¹¹ believes that there exists a reduction in the amount of prothrombin which accounts for the delay in thrombin formation and clotting. However, the experiments of Addis¹² seem more conclusive, showing only a delay in thrombin production, with no deficiency in the total amount finally formed. Mills¹³ likewise concluded that the fault lay, not in the amount of prothrombin or thrombin but in the delayed activation of the former to the latter. He found prothrombin in hemophilia to be much more resistant to activation with cephalin than in normal blood, and that this fault was corrected by the induction of a local skin protein reaction. *Thrombin* of hemophilic blood, when once formed, is normal in amount and action. It possesses the proper capacity for clotting either normal or hemophilic fibrinogen. *Blood fibrinogen and calcium* have been found normal in amount and character by all who have studied them in this condition. The presence of *anticoagulants* in the blood, to account for its delayed clotting, has received much study but again there is almost unanimous agreement. There is no excess antithrombin present^{10 13 14 15 16 17} nor is antiprothrombin increased in amount.^{18 19}

Other pathologic findings in hemophilia usually include marked dental caries with root abscesses, and chronic joint changes. These, however, more properly come under the heading of complications of the disease. The only other pathologic condition found is the entire lack of female sex hormone in the urine of these patients. The work of Birch²⁰ in this connection needs confirmation by other workers and on a larger series of patients.

Symptoms and Signs—Repeated severe hemorrhages furnish the outstanding feature of the disease. These usually start in early childhood, rarely however before two years of age, and continue throughout childhood. Adolescence usually brings a measure of relief, and by early adult life the hemorrhages are in most cases fewer and less severe. The severity of the bleeding lies not in the rate of blood loss, but in its duration. Frequently the loss is only through seepage around clots, but the continuance of this for ten days to two weeks brings the patient down to a critical level of anemia. It would seem that the only spontaneous means of stopping the bleeding in many cases lies in closure of the wound by healing processes rather than through the clotting power of the blood. Bleeding most frequently occurs from the *gums*, with the shedding of the deciduous teeth or tooth extraction, from a *torn frenum* in the mouth, into the joints, especially the knees, elbows, and shoulders, and from *traumatic* injuries to any part of the body. Subcutaneous and intramuscular extravasations from injury are rather common. Probably the most difficult hemorrhages to control are those from the gums and frenum, while those in the joints cause most discomfort to the patient and lead to the greatest harm through the joint changes produced.

There are no characteristic physical findings in hemophilia except those resulting from the bleeding tendency. Because of the danger attendant on extraction of teeth, together with a rather poor state of nutrition in these persons,

dental caries is usually marked and root infections are common. Joint deformities with varying degrees of limitation of motion are common. Anemia is seen of course during and immediately after the hemorrhages but recovery is nearly always prompt once the bleeding has ceased.

The principal sign of the disease is the delayed clotting of the blood with the frequent and prolonged hemorrhages. The clotting time may range anywhere from normal limits up to several hours and varies at different periods as discussed in a previous paragraph. One expression of this delayed clotting is the *prolonged prothrombin time* as described by Howell¹³ and verified by numerous other investigators.^{9, 14, 20, 21} The bleeding time is normal as are also the cellular elements and platelets of the blood.

Course of the Disease—Manifesting itself rarely before the second year of life the bleeding tendency is most severe through early childhood, usually becoming less troublesome toward the beginning of adolescence. By early adult life it often becomes quite dormant or disappears almost entirely. In a few cases it persists in severe form throughout life. In no patient does the disease maintain the same severity at all times. All hemophilic patients have periods when their bleeding tendency is lessened or gone completely. But these periods may suddenly change over into ones of great severity. These periods of varying intensity usually cover weeks or months. Very often in the time of betterment the patient will seek dental or surgical aid without acquainting the dentist or surgeon of his disease and find himself suddenly in the severe phase of hemophilia coincident with the operative procedure. Great caution must therefore be used in carrying out any operative procedures on patients with a positive personal history of hemophilia no matter how little trouble they have had in recent months, nor how normal the blood coagulability has recently been.

Complications—*Dental caries and root infections*, together with the accompanying gingivitis form the most common complications of hemophilia. They should be classed as complications because they usually result from neglect of the teeth and gums through fear of hemorrhage. A mild degree of malnutrition is usually present, whether from the abnormal life led by the person, from oral infection, or from the repeated hemorrhages is difficult to say. Chronic joint changes resulting from repeated bleeding into the joints and the presence of a low grade infection, usually end in hypertrophic arthritis and varying degrees of limitation of motion.

Diagnosis—Diagnosis of hemophilia rests mainly on three points: (a) positive family history as given earlier in this article; (b) personal history of repeated hemorrhages beginning in early childhood; and (c) a prolonged clotting time of the blood. In many cases the family history is negative or lacking and diagnosis is made on the other two points. The personal history of repeated hemorrhages is so important in the diagnosis with patients past early childhood that one should hesitate to class as hemophilic a patient with an initial severe hemorrhage without going into a careful study of the blood coagulability. When presented with an initial hemorrhage in early childhood however diagnosis must often rest entirely on studies of the blood coagulability. In such a case the determination of the clotting time is important. Many methods are available

for this purpose, the simplest of which are probably the test tube and the capillary tube methods. A description of the technic of the latter method, together with its factors of error and the normal fluctuation of the clotting time will be found in an article by Mills and Peterson.²² A marked delay in the clotting time of the blood, but with the eventual formation of a clot which contracts normally and the presence of normal bleeding time and normal number of blood platelets would favor the diagnosis of hemophilia.

Differentiation from other hemorrhagic conditions is of course necessary. The question is most often raised in cases of purpuric hemorrhages, especially the repeated, severe, and protracted hemorrhages of essential thrombocytopenic purpura. Differentiation here depends on the presence in purpura of a normal or slightly prolonged clotting time, an abnormally long bleeding time, a marked reduction in the number of blood platelets and the production of a nonretractile clot. Accurate diagnosis is especially important in those cases of essential purpura in which splenectomy is being considered, since a mistake might well mean a fatal outcome. Acute leucemia in childhood is sometimes accompanied by hemorrhages which require differentiation from those of hemophilia, but here again the picture and diagnostic points are much the same as those for purpura. Severe hemorrhages associated with obstructive jaundice and due to the high bile salt content of the blood, need scarcely be confused with hemophilia. Bleeding of the newborn is often wrongly diagnosed as hemophilia, although such a mistake is easily avoided. This disease, while very similar to hemophilia in the type of bleeding seen, comes on only during the first week of life, while hemophilia very rarely manifests itself before the second year. There exists no known relationship between these two hemorrhagic diseases. Hemolytic streptococcal infections, particularly in the sockets of extracted teeth, may lead to such prolonged bleeding as to raise the question of hemophilia. None of the typical diagnostic points are to be found, however, and differentiation should be easy. Prolonged malnutrition in children at times brings on a bleeding tendency, but it is usually more of the purpuric type.

Prognosis —The outlook for the hemophilic child in past decades was distinctly unfavorable. The mortality from hemorrhage was high during the years of childhood, while the nutritional state and physical development were usually retarded by the abnormal life of constant care and fear of hemorrhage. Later in life the repeated joint hemorrhages and low grade infection left these unfortunate persons with such deformities and limitation of motion that most of them remained semi-invalids for life. Only too frequently the betterment of bleeding tendency that came with adolescence and adult life was more than counterbalanced by the increase in joint distress from the more active life. In a great many cases, fortunately, the disease can be expected to decline in severity from the beginning of adolescence onward, so that its handicaps may eventually be outgrown, except for the joint involvements.

In more recent years the prognosis has assumed a much more hopeful character. Several new methods of treatment have robbed the hemorrhages of much of their danger, while better knowledge of nutrition and of its importance in this disease has made possible a more normal childhood development. In fact,

if the plan of treatment as outlined below is followed consistently, hemophilia should cease to be such a dreaded disease and such a scourge to its victims

Treatment—The treatment of hemophilia is to be divided into two parts (1) the handling of the immediate hemorrhage and (2) prophylactic measures for lessening the future hazards of the disease. In the treatment of the immediate hemorrhage too much emphasis cannot be laid on one important point *no time should be lost in applying the most effective means of hemostasis at hand*. Only too frequently it happens that little attention is paid to the slow blood loss from these patients for the first two or three days, the hope being that it will stop spontaneously. Due to the fact that prolonged blood loss often results in a hemorrhagic phase associated with the anemia and reduction in concentration of the blood clotting constituents it is very important that prompt and energetic measures be instituted early in hemophilic hemorrhages. The first two days offer the greatest chance of prompt stoppage of the bleeding. Beyond this period great difficulty may be encountered and repeated blood transfusions required.

In handling a hemophilic hemorrhage the first step is of course, the application of physical means of compression (packing, bandaging surgical ligations, etc.) to slow the blood loss as much as possible, but at the same time instituting those forms of treatment best adapted to increase the coagulability of the blood. Many hemostatic and coagulant preparations have been marketed for this purpose, a few of which are sometimes effective. In general those preparations depending only on cephalin for their effectiveness are not of much value in hemophilia, while those containing tissue fibrinogen, such as the lung, brain or other tissue extracts, are more useful, either when given orally or subcutaneously. I personally favor the purified tissue fibrinogen rather than any of the crude tissue extracts. If administered orally tissue fibrinogen should be given in 3 to 5 c c doses (of a 15 per cent solution) in cold water on an empty stomach, preferably one-half hour before meals and at midnight. This is necessary in order to approximate a continuous effect. Subcutaneously 1 to 2 c c doses should be given every eight hours for a continuous effect. In the beginning it is well to give the injections every two hours for four injections before going to the eight-hour schedule. This gives a maximum effect in a minimum time. Administration of the coagulant should be continued for at least twenty-four hours after the bleeding has ceased. These same principles of administration would hold for any of the biologic coagulants in common use.

Other procedures also to be instituted at once include the administration of a high protein diet, as will be discussed later the induction of a local skin protein reaction, provided the patient has been previously sensitized and the subcutaneous injection of 0.1 to 0.3 c c of adrenalin solution every four hours except in cases where the bleeding is arterial. In case a local skin protein reaction can be elicited this is usually sufficient to stop the bleeding without the use of tissue fibrinogen or adrenalin. In every other case, however all the other procedures previously mentioned should be used promptly and in conjunction so as to avoid the greater difficulties that come with the prolonged blood loss.

In case the patient is seen for the first time only after he has been bleeding

for a number of days, then the procedure already mentioned should be begun at once, but with less hope of benefit. In this case one should prepare for blood transfusion as soon as possible. Repeated transfusions are often necessary at this stage, and should be used always in conjunction with the other procedures previously mentioned. One caution should be kept in mind, however. Transfusion should not be performed on a patient within eight hours after a previous dose of tissue fibrinogen since this coagulant tends to intensify the transfusion reactions that sometimes occur. Tissue fibrinogen may be administered with safety, however within an hour after the transfusion. It seems to make little difference whether the direct or the citrate method be used in performing the transfusion. This procedure, which is often a life saving measure in the prolonged hemorrhages of hemophilia, is not justified during the first two days of bleeding. Other treatment is usually effective during this early stage and is more economical. It is always advisable to keep transfusion as a reserve measure as long as possible, since the patient so often considers his condition more critical when he learns that the physician is planning to resort to transfusion.

Treatment of hemophilic patients in a prophylactic way involves several separate procedures which are here detailed.

1 *Sensitization to a foreign protein* offers such an immediately effective means of treating hemorrhages when they occur, and of preventing their occurrence, that I give it first place in prophylaxis. Vines²³ discovered this method, and its effectiveness in increasing the coagulability of hemophilic blood has since been verified by the writer¹⁵ and others.^{6, 24-26} It is effective in such a large percentage of hemophiliacs that no physician handling these patients is justified in failure to make proper use of it. The technic is as follows. The patient is sensitized to some foreign protein by intramuscular injection, I prefer sheep or hen serum, since they have practically no other therapeutic use. Three or four cubic centimeters of the serum are injected intramuscularly after making certain that the patient is not already sensitive to a drop administered intradermally. After the expiration of two weeks, intradermal injection of a drop of the serum is again made. The formation of the usual urticarial wheal denotes proper sensitivity, and studies of the blood coagulability before and after the induction of the local reaction will show a marked improvement. Not only is the clotting time much shortened, but one now finds the prothrombin to be much more nearly normal in its reactions, especially toward cephalin.¹⁶ Should the clotting time not shorten sufficiently after the first skin reaction, one may repeat the intracutaneous injection for several successive weeks in different skin areas. Repeated local reactions do not tend to reduce the general sensitivity which usually lasts for a year or more and which may be subsequently renewed using the same or a different protein. The production of a generalized systemic protein reaction is to be carefully avoided, since its effect is to produce a temporary hemorrhagic state which might prove disastrous to the hemophilic patient. Care should therefore be taken to make sure that the patient is not already sensitive to the serum to be injected intramuscularly. Likewise, it would be unfortunate if intended intradermal injections should be given subcutaneously instead. By

keeping all one's hemophilic patients sensitive to some foreign protein at least during the time in life when their disease is most troublesome one always has at hand an immediately effective method of treating hemorrhages as they occur. And if one desires to use the method for the prevention of bleeding one needs only to induce the skin reactions as often as is indicated by observations of the blood coagulability.

2 The use of *high protein diet* is an important adjunct in prophylaxis as well as in treatment of the hemorrhages. It is definitely established that the absorption of protein food from the intestine is accompanied by an increased coagulability of the blood so that the clotting time is usually shortened by 30 to 40 per cent for one to four hours after each meal containing protein.^{26 27} Use has been made of this fact in the treatment of hemorrhagic states with considerable success.^{28 29} It involves the administration of a moderate amount of protein in some form at each meal and the drinking of milk, cocoa or egg nog between meals and at least once during the night. In this way the protein effect on blood coagulability is continuous and tends to be cumulative. A high carbohydrate low protein diet has just the reverse effect on the blood and should be avoided in hemophilia. There are practically no contraindications to this diet in hemophilia, so that use should always be made of it for prophylaxis or treatment of hemorrhages. Should it give rise to too much intestinal putrefaction, relief can at once be obtained from the use of the following prescription:

	Kaolin	vi
	Aq. dest.	iv
	Syr. Tolu. q. s.	℥ii
M. Sig.	One half ounce each evening for adults	
	One drachm each evening for children	

3 *Theelin*, the follicular hormone now on the market, has been found by Birch³⁰ to be very effective in hemophilia. Wiedemer, in Cincinnati, has had occasion recently to verify the findings of Birch in this regard. The technic she has found most effective in adolescent patients consists in subcutaneous injections of 1 cc. of theelin (Parke, Davis & Company) every second or third day. Within two or three weeks the clotting time usually has approached close to normal and can be kept there by two injections a week. Daily injections seem to be too frequent as the clotting time tends to rise. In like manner weekly intervals are too long. There is no permanency to the effects since the clotting time promptly begins to mount once they are discontinued. Coincident with the disappearance of the bleeding tendency under theelin there usually occurs an improvement in nutrition with a gain in body weight. It is obvious that this treatment is not applicable for the arrest of an existing hemorrhage on account of the long latent period. Its main usefulness will be found in the preparation of these patients for tooth extraction or minor surgical procedures and for use when a patient appears to be entering a hemorrhagic period. Its usefulness may of course be considerably increased as our knowledge of it grows with experience.

4 *Improvement in the nutritional state* of hemophilic patients is one step that should always receive careful consideration in outlining a plan of treatment.

Proper diet should be provided keeping in mind the protein effect. Especial attention should be given to the vitamin content of the diet, particularly the vitamin B content (old nomenclature). A good adjunct for this purpose is the use of purified wheat hearts, such as may be obtained very cheaply from most milling companies. Such a product may easily be incorporated in the preparation of pancakes, bread, cooked breakfast cereals, etc. It is pleasant to use and provides an economical supply of important vitamins. There are much more expensive preparations on the market which one may use for this same purpose, if it is desired. Further stimulation of the metabolism by ultraviolet light or heliotherapy is helpful at times during the winter and spring months. One's effort should be directed mainly toward securing as nearly normal physical development in these children as is possible. As their nutritional state improves, so also does their bleeding tendency, in most instances.

5 *Removal of foci of infections* should receive careful attention for two reasons: first, because it will greatly aid in the desired nutritional improvement, and second on account of its relation to the chronic arthritis so frequently present in these patients. Carious teeth and infected roots form the greatest danger in this regard. The fear of hemorrhage so often prevents these patients seeking or receiving proper dental care, with the result that advanced stages of infection and decay are usually present. It is altogether likely that it is these foci of infection, seeding into the hemorrhagic joints time after time, that prevent proper recovery following each joint hemorrhage and eventually lead to the bad deformities so often seen. With our present methods of preparing these patients for dental work, little difficulty should be encountered in eradicating foci of infection from the mouth. Infected sinuses should likewise receive proper attention, although surgical operations on the sinuses or tonsils should still be undertaken with a great deal of caution, no matter how well prepared the patient may appear. Restoration of joint function becomes an orthopedic problem only after all evident foci of infection have been removed and the patient's blood coagulability brought as near to normal as is possible by the previously described means.

One should bear in mind that the prophylactic measures outlined here do not conflict with one another, and may well be used in conjunction, especially if any operative procedures are contemplated. As previously stated, one need have little dread of hemophilia if the treatment measures given here are properly used. Since the best of them are of very recent application, more experience will be necessary before one can be certain just how completely these patients can be prepared for the surgical emergencies that at times arise.

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VARIATIONS IN THE DIAMETER OF THE GRANULOCYTES*

A PRELIMINARY STUDY

J W LOVE, M D CLEVELAND, OHIO

SINCE the epochal discovery by Ehrlich¹ that blood smears could be stained with the analine dyes and the modification of these stains by Jenner,² Pappenheim,³ Romanowsky,⁴ Giemsa,⁵ and Wright,⁶ increasing interest in the stained elements of the blood has been shown. From the pioneer work of these men there has come an increasing accuracy in the field of clinical hematology.

The object of the present study was to determine the diameter of the granular elements of the blood, and the normal distribution curve, to establish the mean average diameter of these cells as seen in stained smears, to note the variations from normal, and finally to see if any clinical application or correlation could be made of these conclusions.

Credit for the discovery of the white cell is generally given to the English physiologist, Hewson,⁷ whose published work appeared in 1771. Little note was taken of these cells, however, until Schultze⁸ in 1865 studied warm-stage preparations, differentiated the various types of white cells and gave us the first accurate measurements of them. It was about fifteen years later that Ehrlich added immeasurably to the vista of hematology by the use of analine dye stains for the study of blood films.

In all the work reported in this paper smears were made upon cover slips and stained with the familiar Wright's stain plus a buffer made of sodium and potassium phosphates as initiated by McJunkin.⁹ The cover slips were mounted on slides with gum damar and the actual measurements were made by using a filar micrometer eyepiece. The calibration with the microscope drawtube at 160 mm. 1/12 mm. Zeiss lens oil immersion objective, was so standardized that 11 drum divisions measured one micron, that is, each drum division was equivalent to one-eleventh of a micron. Only those cells were measured which were entirely round, halo free, and had assumed the familiar "rounded-up" appearance in the film, those cells the outline of which was oval or otherwise distorted or deformed were particularly avoided as were the edges of the smear where malformations of the leucocyte are so often discovered.

Many authors give various figures for the size of the granulocyte (see Table I). Wright⁶ in Nelson's Medicine gives 9 to 12 microns as the size of the polymorphonuclear granulocyte, no figure for the eosinophile, and 8 to 10 microns for the size of the basophilic cell. Jordan¹⁰ says that the neutrophiles "range in size from 7.5 μ to 10 μ in diameter" and that the eosinophiles and basophiles are "slightly smaller." Maximow,¹¹ Bailey,¹² Halliburton,¹³ and others give various other figures. None of these authors give any method for

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arriving at these conclusions or quote any references concerning them. Valuable references are to be found, however in Bunting's¹¹ article in *Cowdry's Special Cytology*, Hoeber, 1928. Bunting states "The range of variation in diameter (of neutrophiles) given by Schultze and commonly accepted is from 9 to 12 μ ."

TABLE I
SIZE OF GRANULOCYTES AS REPORTED BY VARIOUS AUTHORS

AUTHOR	NEUTROPHILES	EOSINOPHILES	BASOPHILES
Bailey	8 12 μ	12 14 μ	8 10 μ
Barker	9 12 μ	sl larger	8 10 μ
Bunting	9 12 μ	10 12 μ	8 10 μ
Cummer	9 12 μ	sl larger	8 10 μ
Halliburton	9 12 μ	12 15 μ	10 μ
Jordan	7 5 10 μ	sl smaller	sl smaller
Maximow	10 12 μ	12 μ	10 μ
Ordway and Gorham	9 12 μ		
Wright	9 12 μ		10 μ

The present study has for its basis the measurement of the diameters of one hundred or more polymorphonuclear granulocytes in each of fifteen normal individuals, this number being chosen in order to insure measurement of a sufficient number to establish a normal. Of these fifteen normals eight were men and seven were women. These individuals were unselected except for general good health and no account was taken of age, size, or body weight. Naturally the figures obtained apply only in this climate.

It was found that there is no distinct difference in diameter of cells according to sex. The average diameter of the polymorphonuclear granulocytes for the series was 13.3 μ . None of the normals had a leucocytosis or a leucopenia. The diameter of many of the cells found was greater or less than the average diameter of 13.1 μ , but in series they remain remarkably close to this figure and it may be taken as a constant. The smallest polymorphonuclear cells were 8.8 μ and 9.1 μ in diameter but only one specimen of each of these was found, correspondingly, cells of 15 μ in diameter occurred several times but were distinctly rare in smears of normal blood.

The absolute spread of these cells therefore is from 8.8 μ to 15.8 μ , but these figures are outside the usual limits found and the great majority show little variation. In typical distribution curves the cell diameters range from 11.2 μ to 15.0 μ and these latter figures are used in the construction of distribution curves to represent the normal variations in diameter (see Chart 1).

The diameter of the normal eosinophile in stained cover slip preparations taken from a somewhat larger group of normals in order to count one hundred such cells was found to be 14.3 μ . This is the average diameter of 100 normal eosinophiles and no smear of an eosinophilia was included. The size of the normal basophile is based upon the measurement of 25 normal specimens of this cell and was found to be 13.2 μ for adults of the series. The range in diameters for the eosinophile was from 12.2 μ to 16.7 μ , for the basophile 10.9 μ to 14.6 μ .

It was thought advisable to give a figure for the diameter of the normal non-filamentous polymorphonuclear cell, since this has been the subject of recent studies and to see that it did not vary consistently from the size of other polymorphonuclear cells. The average diameter of 100 such cells selected at random from normals was found to be 13.0μ .

The normal diameters for leucocytes of this series therefore are as follows: polymorphonuclear granulocytes 13.1μ , eosinophiles 14.3μ , and basophiles 13.2μ , these are accepted as constants in order to compare with the diameters of the cells measured in the abnormal conditions to follow.

The first of these conditions in which the diameter of the granulocyte was measured was *leucocytosis*. Five cases were chosen at random. They represent some of the ordinary conditions in which we have come to expect an elevation of the leucocytes to occur and represent leucocytosis in varying degrees ranging from a moderate elevation of 9,700 to that of a rather severe degree, 45,000 white

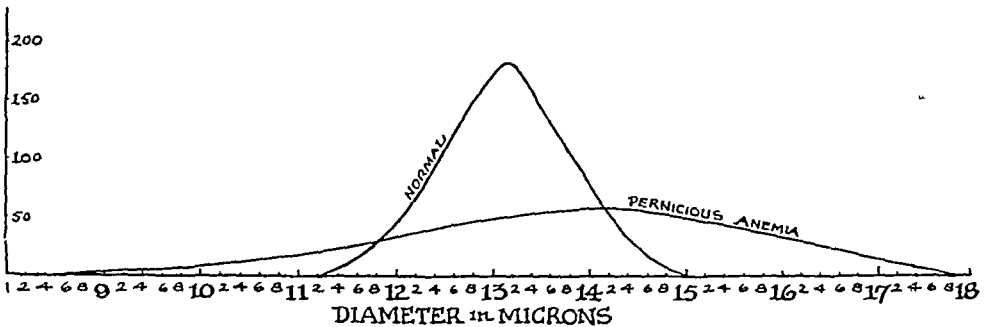


Chart 1

cells. In these conditions the polymorphonuclear cell was present in predominance, 82 per cent to 96 per cent (Table II).

TABLE II
DIAMETER OF POLYMORPHONUCLEAR GRANULOCYTES IN LEUCOCYTOSIS

CASE	DIAGNOSIS	LEUCOCYTOSIS	DIAMETER
1	Infectious arthritis	17,000	13.5μ
2	Sinusitis	20,000	13.1μ
3	Pylitis	25,000	13.1μ
4	Bronchopneumonia	33,000	13.0μ
5	Lobar pneumonia	45,000	12.8μ

The average diameter of the polymorphonuclear cell in these conditions is 13.0μ which is so close to the normal figure as to be without significance. One would believe, therefore, that stimulation of the bone marrow in the customary degree causes no appreciable change in the diameter of the granulocytes no matter how rapidly they are added to the blood stream.

The second abnormal condition to be investigated was *leucopenia*. In one case in which the total white count was 2,300 cells resulting from multiple abscess formation the average white cell diameter was nearly normal, 12.7μ . Three cases of malaria in which the total counts were 1,600, 1,700, and 2,300

white blood cells respectively had average cell diameters considerably below the normal figure, the first being $11.6\ \mu$, the second $11.5\ \mu$, and the third $11.3\ \mu$. A case in which the total white cell count was 500 was seen in a condition which resembled malignant neutropenia, but too few cells were observed to make proper measurements and no other suitable case has been seen. Although further work must be done on the group of leucopenias, nevertheless, one might justifiably reach the conclusion that depression of the bone marrow results in the production of a granulocyte of somewhat smaller diameter than normal, especially as seen in malaria. The leucopenia accompanying pernicious anemia will be considered later.

The third abnormal condition to be studied was *polycythemia vera*. Recently some interesting work has been done by Cross¹² on the white cells in this condition. The diameter of the white cells was measured in three cases at this Clinic. All these cases showed an increase in red blood corpuscles and hemoglobin, and an increased blood volume was found by actual measurement. The average diameter of the polymorphonuclear white cells was found to be $13.1\ \mu$, nor did it vary from this although the number of white cells ranged between 8,500 and 20,900. No case in which a marked leucopenia was present was at hand for study.

Fourth, the white cell diameter in the condition which is commonly designated simple *achlorhydric anemia* was investigated. In studies in five typical cases no significant change from the normal was noted. This result might have been predicted inasmuch as the pathologic physiology in this condition seems to concern hemoglobin anabolism rather than bone marrow deficiency.

Fifth, in addition to the three cases of *polycythemia vera* showing variation in the diameter of the red blood corpuscles, one case of hepatic disease was seen which showed a marked *megalocytosis* and the diameter of the white blood cells was accordingly measured. Here again, however, no change from the average diameter of $13.1\ \mu$ was found.

Hence, it may be seen that the diameter of the white blood cells is a very stable measurement and in practically all the above pathologic conditions, with the exception of malarial leucopenia, no change in diameter could be found. It might be added at this point that the number of lobules of the nucleus seems to bear no relation to the diameter of the cells in any of the measurements encountered.

Sixth, cases of *pernicious anemia* were studied both before and after treatment with a view to establishing whether the variations in size which the red blood cells are known to undergo might be paralleled in the white blood cells. Routine measurements were made in the case of patients as they presented themselves at the Clinic. They were all suffering from various grades of anemia and all showed some depression of their hemopoietic activities, hence all the measurements in the beginning were actually made on leucopenias. The white blood cell count ranged from a total of 1,600 cells up to 2,500 cells. In ten typical cases it was found that the diameter of the polymorphonuclear granulocyte was markedly increased, $14.0\ \mu$. This increase is striking in some cases; individual cells of $17.5\ \mu$ to $18.0\ \mu$ in diameter were not uncommonly observed.

There are, however, cells of greatly diminished diameter, as of $85\ \mu$ to $90\ \mu$. Thus the spread of white blood cells in pernicious anemia is quite wide (broad base to the distribution curve) ranging from $85\ \mu$ to $180\ \mu$. These variations are shown graphically in Chart 1.

Five cases of pernicious anemia were studied both before and after the red blood cell count and hemoglobin had risen from quite a low figure to approxi-

TABLE III
AVERAGE DIAMETER OF WHITE BLOOD CELLS IN PERNICIOUS ANEMIA

CASE	BEFORE TREATMENT	AFTER TREATMENT
1	$143\ \mu$	$131\ \mu$
2	$138\ \mu$	$131\ \mu$
3	$142\ \mu$	$134\ \mu$
4	$140\ \mu$	$128\ \mu$
5	$140\ \mu$	$133\ \mu$
Averages	$140\ \mu$	$131\ \mu$

PERNICIOUS ANEMIA GRANULOCYTES

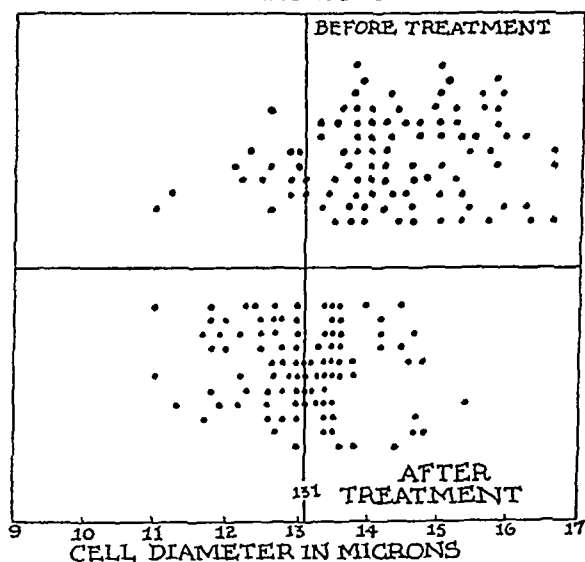


Chart 2

mately normal (Table III). The white cells were found to be large at first, averaging $140\ \mu$ in diameter but returned to their normal figure when improvement had occurred. This condition of the white cells has been discovered only in true Addisonian anemia and not in any other type of disease so far encountered. Although this increase in the diameter of white cells, megalopolcytosis, does not seem great, it is present in all the cases studied and will perhaps constitute another factor in enabling us to understand this malady and perhaps throw some light upon what occurs in a remission. This change from abnormal (increased) diameter to normal is shown graphically in one case in Chart 2.

SUMMARY AND CONCLUSIONS

Measurement of the diameter of white blood cells in stained blood films in the case of fifteen normal adults shows the mean average diameter of the cells to be 13.1μ for polymorphonuclears 14.3μ for eosinophiles and 13.2μ for basophiles. These figures are well above the commonly quoted normals which seem to be based upon the work done by Schultze in 1865. For comparative studies on changing white cell diameters these figures may be considered as constants.

The variation in diameter (spread) of the normal polymorphonuclear granulocyte is within the limits of from 11.6μ to 14.6μ usually. Contrary to this, pernicious anemia is accompanied by a granulocyte of larger diameter than normal and the spread in distribution curves of untreated cases is greatly increased, ranging from 8.5μ to 18.0μ for polymorphonuclear cells. During a remission these cells return to their normal diameter.

Significant variations from the normal white cell diameter occur only in certain abnormal blood states and no change is found in the ordinary leucocytosis or leucopenia.

Variations of rather marked degree do occur, however, when there is marked depression of the bone marrow as in the leucopenia accompanying malaria and pernicious anemia.

No explanation for these phenomena is offered at this time.

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EDITORIAL

The March of Hematology

JOHN HUNTER¹ in his Treatise on the Blood, remarked that the early observers of the blood cells "probably imagined more than they saw." Hunter's book testifies as to the scant knowledge of the blood in his time, since his discussions centered largely around the coagulation, the buffy coat, the cause of the difference in color of venous and arterial blood and similar topics. The development of hematology as we now know it began in the middle of the nineteenth century when Vieillot² in 1851, first enumerated the erythrocytes and thus stimulated work in blood counting. The original crude and laborious methods were rapidly improved so that the counting of cells soon became a clinical procedure. Malassez³ monograph on the enumeration of the red cells appeared in 1873. The counting chamber devised by Gowers⁴ in 1877, remained the standard until recent years.

Hemoglobin was discovered and isolated in crystalline form by Funke⁵ in 1851, and Welch⁶ in 1854 made the first clinical estimation of hemoglobin by

comparing a fixed dilution of the unknown blood with dilutions of normal blood. Interest in hemoglobin was widespread and quite complete analyses of hemoglobin crystals soon were made. Accurate knowledge of hemoglobin is due largely to the early work of Hoppe-Seyler⁷ who showed that hemoglobin combines with oxygen in the lungs to form oxyhemoglobin which in turn gives up the oxygen in the tissues again to form hemoglobin. He thus identified the fundamental importance of the blood pigments in tissue metabolism. Peyer's⁸ monograph on the blood pigments appeared in 1871. The first monograph on clinical hemoglobinometry by Leichtenstern⁹ was published in 1878 and a number of hemoglobinometers were suggested before 1880.

With the development of accurate methods for the counting of the blood cells and the determination of hemoglobin, clinical applications were made quickly. In 1864, Welcher,¹⁰ a student of Vierordt's, wrote a very comprehensive article on the "size, the number, the volume, the surface, and the color of the blood corpuscles of man and animals." The first clinical hematology by Hayem¹¹ appeared in 1878. Soon afterwards, (1883) Laache's¹² classic monograph on anemia was published. The names of Vierordt, Welcher, Hoppe-Seyler, Malassez, Hayem, and Gowers are closely identified with the discovery of the fundamentals of hematology.

The next important development was the introduction of Ehrlich's¹³ method for staining blood film with anilin dyes.¹³ This opened an entirely new field for investigation of the blood and made possible the morphologic study of the different types of cells. The final chapter in the development of fundamental methods for blood study was Hedin's¹⁴ suggestion concerning the use of centrifugal force to separate cells and plasma in the hematocrit.

Since then many new refinements in blood study have been and still are being added. We now constantly use reticulocyte counts, determine the bile pigments, utilize knowledge concerning the embryology of the blood, measure cells, differentiate cells by supravital, oxidase and many other stains, and employ numerous procedures almost routinely in the study of blood. Any known blood dyscrasia can be most carefully investigated from the laboratory standpoint, if one but uses the measures now available.

In no field of medicine today is more intensive investigation being made or is more rapid progress resulting than in the acquisition of new knowledge concerning normal and pathologic conditions of the blood. The medical journals contain many articles on every phase of the subject and many papers are contributed to the programs of the various societies. This added interest is due in large measure to improvements in methods of treatment of the anemias and of other blood diseases for which credit is due largely to Whipple and to Minot and Murphy. Much remains to be done. The leucemias and some other blood dyscrasias continue a challenge in regard to treatment. Every worker in this important field, however, can well be proud of the march of hematology.

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R L II

CORRESPONDENCE

Dear Sir

In my paper on Bacteriophage in *Clinical Medicine* which appeared in the April number of the *Journal of Laboratory and Clinical Medicine*, I made the following statement

"The early experiments of d'Herelle with fowl typhoid were not sufficiently extensive to be conclusive His later work with birbone in buffaloes was apparently satisfactory although Cowles and Hale make the statement that in a personal communication d'Herelle claims the opposite"

This statement is misleading since the work to which Cowles and Hale referred concerned therapeutic application, the results of which had not been published owing to the small number of animals concerned The work which d'Herelle reported concerned immunization of buffaloes and it has been amply confirmed by LeLouet Hence, there has been no change in the published papers of d'Herelle on this subject as was implied in my statement

I would greatly appreciate it if you could find space for this correction in a forthcoming number of the *Journal*, also for the statement of my regret that there should have been a misunderstanding on my part of the statement of Cowles and Hale

(Signed) N W LARKUM

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No 10

SYMPOSIUM ON HEMATOLOGY

(Concluded from June issue)

STUDIES ON THE STRUCTURE AND FUNCTION OF BONE MARROW*

I VARIABILITY OF THE HEMOPOIETIC PATTERN AND CONSIDERATION OF METHOD FOR EXAMINATION

R P CUSTER, M D, PHILADELPHIA, PA

THERE is perhaps less consistency in the study of the bone marrow than of any other tissue of the body removed at autopsy for the study of diseases of the hemolytopoietic system. This is especially unfortunate and probably results from the difficulties encountered in removal and preparation. Not only is the tissue scattered widespread throughout the body and encased in a bony envelope that is difficult to remove, often necessitating an extra body incision, but, as will be shown later, its cellular state varies (more than any other tissue) in different localities, both in different bones and at different levels of the same bone, even a single cross section at a given level shows wide inconsistency. Gross examination is often fallacious in that congestion or hemorrhage may be indistinguishable from hemopoietic cellular hyperplasia. There is thus a considerable need for a standardized method of bone marrow study.

That the marrow has not been studied either sufficiently or efficiently during the course of routine autopsy work is shown by an analysis of the postmortem services of two teaching hospitals in this city. During the years 1927, 1928, and 1929, 2618 autopsies were performed. In this series, the bone marrow was examined 47 times, or in 1.7 per cent of cases. In 25 of these, the marrow of but one bone was observed, several instances simply grossly. For the most part the histologic descriptions merely confirmed the gross diagnosis of hyperplasia or normality, no mention of cytology being made. Examination of the slides showed that in many cases cytologic detail was extremely poor.

*From the Department of Pathology of the School of Medicine, University of Pennsylvania and The Laboratories of the Philadelphia General Hospital, Philadelphia.

It is a well-known fact that during early childhood the marrow of practically all of the bones is of the cellular type and that this condition persists in the vertebrae, ribs, sternum, bones of the skull, and os innominatum, and to some extent in the proximal epiphyses of the femur and humerus, throughout life

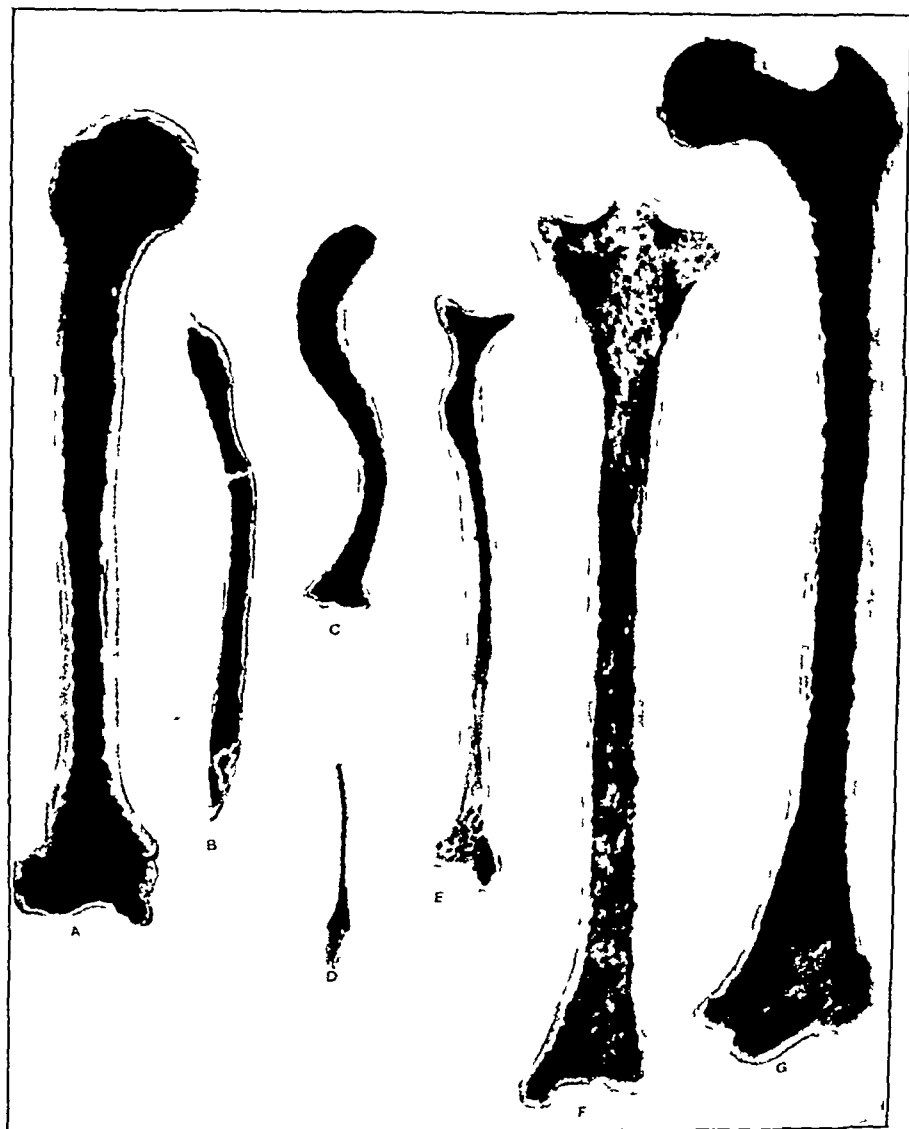


Fig. 1—Characteristic bone marrow in pernicious anemia. *A*, left humerus. *B*, sternum. *C*, right clavicle. *D*, right fifth rib. *E*, right radius. *F*, left tibia. *G*, right femur. (The red of the hyperplastic marrow is here reproduced in black.) (Photographic reproduction of a color plate appearing in 'Pernicious Anaemia and Aplastic Anaemia' by Arthur Sheard through courtesy of Wilham Wood and Company American publishers.)

(Neumann¹) Under normal conditions the shafts of the long bones assume the predominantly fatty and nearly acellular type of marrow beginning at the fifth to seventh year and becoming complete about the eighteenth year. This fat tissue, however, is in an extremely labile state and is capable of quite rapid replace-

ment by hemopoietic elements, beginning usually at the margin of the myeloid cavity and extending to the center as the need arises. I have observed in pigeons under experimental conditions the metamorphosis from an almost completely fatty marrow to a totally cellular marrow in the course of two days. I have seen



Fig. 2.—Bone marrow in pernicious anemia. Showing marked variation in hyperplasia not only in different bones but at different levels of the same bone. The tibia is completely aplastic. A right femur B, left tibia C right radius D right clavicle E right fifth rib F sternum G, left humerus. (Photographic reproduction of a color plate appearing in *Pernicious Anemia and Aplastic Anemia* by Arthur Sheard through courtesy of William Wood and Company American publishers.)

almost as rapid hyperplasia in dogs made anemic by injection of pyridin. This hyperplasia of hemopoietic tissue does not occur with any degree of regularity as mentioned previously. Two cases of pernicious anemia described by Sheard,² in which extensive examination of the marrow was made are particularly illustra-

tive of this fact Fig 1 pictures the more usual findings in an untreated case sometimes even a more solidly cellular marrow being found in the tibia and radius than is shown here In contrast, Fig 2 presents the marrow from a parallel case with equal need for blood cells in which the entire tibia and many levels

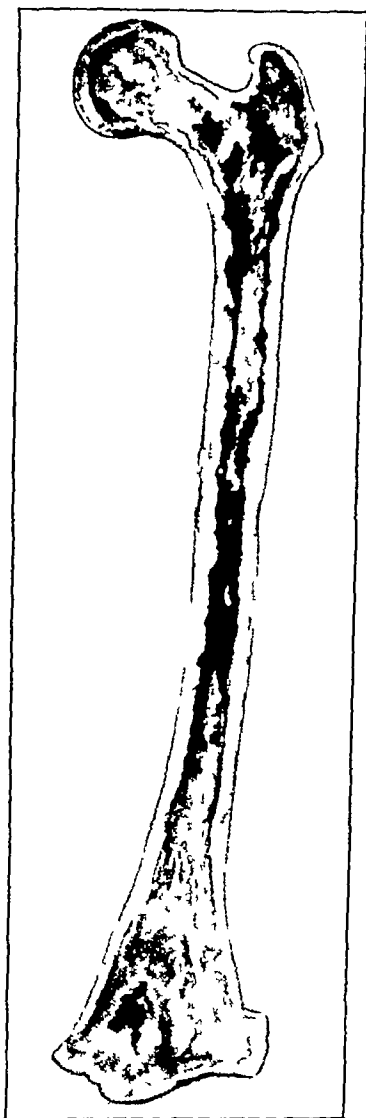


Fig 3—Bone marrow in secondary anemia Showing wide variation in hyperplasia of the femoral marrow at different levels and parts of the cross-section (Photographic reproduction of a color drawing)

of the other bones are nearly acellular Had the tibia or the distal end of the femoral shaft alone been examined in this case, an erroneous conclusion would have been reached, perhaps throwing the case into the category of the aplastic anemias Surprisingly, in the latter case, the proximal epiphyses of the humerus and femur, normally cellular, are seen grossly to be aplastic, this finding having

been confirmed histologically. Fig 3 (author's case) shows the typical irregularity of distribution of blood-forming tissue in secondary anemia of moderate grade. Fig 4, a low-power photograph of a longitudinal section of femoral marrow from a case of carcinomatosis with cachexia, pictures an abrupt line of demarcation between an 80 per cent hyperplastic marrow and a gelatinous degeneration.

Strumia,³ in a report of several cases of myelogenous leucemia, has made pertinent observations on this question. In one of his cases "the bone marrow of both tibiae, upper and lower portions, was yellow, fatty, and contained spongy bone. It appeared altogether as nonfunctioning adult bone marrow. The marrow of the bones of both feet was identical in structure and appearance with the bone marrow of the tibia. The bone marrow of the femur and of the sternum was red and gelatinous with few hemorrhagic points." Carefully prepared sections and smears of each of these bones showed those appearing grossly fatty to have few cells grouped in small foci, while the femur and sternum were hyper-

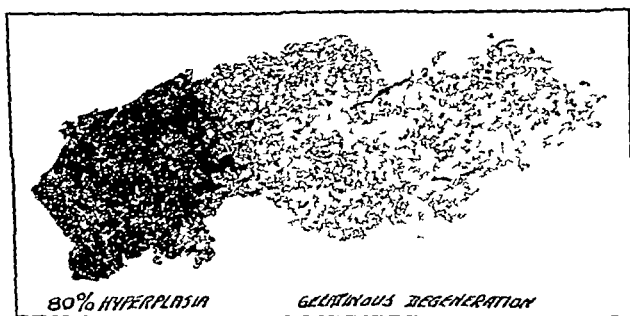


Fig 4—Longitudinal section of femoral marrow ($\times 15$). From a case of carcinomatosis with cachexia showing an abrupt line of demarcation between an 80 per cent hyperplasia and gelatinous degeneration.

plastic. Differential counts showed approximately the same percentages in each bone. Strumia states further, "Aplastic bone marrow in the tibia accompanied by hyperplastic changes in other bones has been observed by the author in a long series of cases including all forms of anemia, acute and chronic leucemia, and acute and chronic inflammatory processes, especially sepsis, accompanied by profound changes of the blood picture. It appears, therefore, that the bone marrow of the tibia is a poor index of the general conditions of the bone marrow. Since the bone marrow of the tibia is readily accessible, it has been often depended upon at autopsies to indicate the general condition of the bone marrow, a practice which should be discontinued."

Confirmative of this latter observation by Strumia are photomicrographs taken from one of our recent cases (Figs 5 to 9 inclusive), a case of bacterial endocarditis with marked secondary anemia. Particularly striking is the difference in cellularity between two levels of the femur 1 centimeter distant from each other. Hemopoietic tissue in the tibia is nonexistent. The sternum and rib each show an increased cellularity over their normal adult state.

The inconsistency in the tissue itself should be sufficient indication for a general and uniform technique and a survey of more than one bone. On the con-

tiary however, except when performed by those workers carrying out special studies on the bone marrow, the reverse is too often the case. For example, the tissue selected for study by many pathologists is removed at random from any one convenient bone, perhaps the tibia on account of its availability. Often one notes in protocols that the bone used is not even mentioned. Fixation in 10 per cent unneutralized formal is usual. The presence of bone spicules makes it necessary to subject the tissue to destructive decalcification. The marrow is then embedded in paraffin, cut at too great a thickness, and stained with hematoxylin and eosin. Consequently, the sections are unsatisfactory for study and often meaningless. Disappointing results naturally lead to a neglect of the tissue.

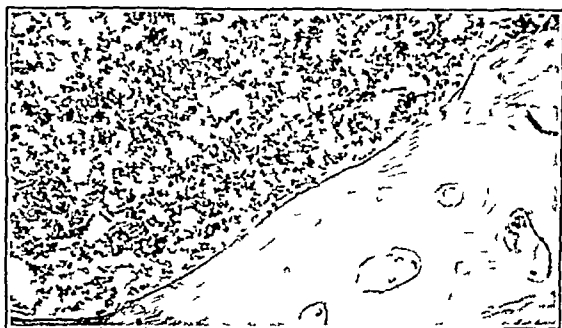


Fig 5

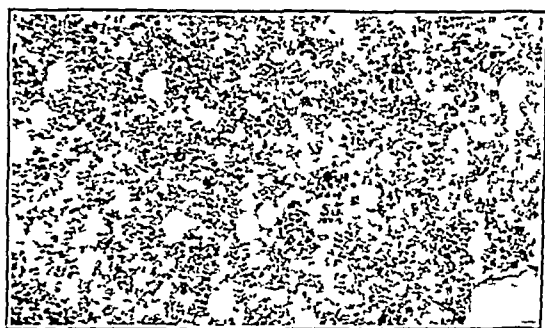


Fig 6

Figs 5 and 6—Bone marrow from case of bacterial endocarditis, showing almost complete cellularity. Fig 5—Rib marrow. Fig 6—Sternal marrow.

METHODS

The technic that has been found most satisfactory and that is productive of most uniform results is outlined in rather brief form as follows:

Removal of Tissue—If but one specimen of marrow is to be taken, the mid-femur is the bone of choice. Expose the midportion of the shaft through a horseshoe incision, through skin, fascia, and muscle, make parallel saw cuts halfway through the bone about three inches apart, and chisel off the cortex between the two cuts (being careful not to break the bone completely through). The marrow cavity is thus exposed and the pencil of marrow may be lifted out intact with a gouge or similarly curved instrument. This will give sufficient tissue for

both cross and longitudinal sections through the marrow. The portion selected for fixation and embedding should be free of bone spicules if possible. Marrow from the tibia may be removed in a similar manner from the middle of the shaft, although the piece need not be so large. The sternum specimen should be taken through the gladiolus between transverse cuts not over five millimeters apart. Care must be exercised in taking rib marrow, as undue pressure on the bone will squeeze the tissue from the medullary cavity. It is best to cut a piece of the rib

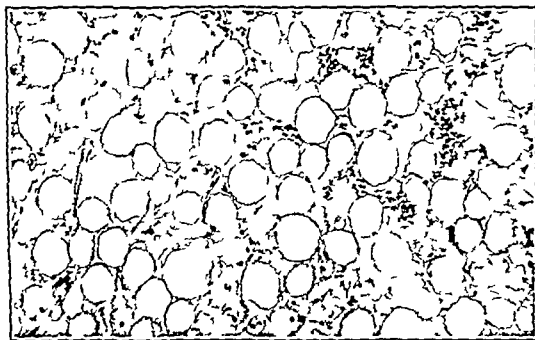


Fig 7—Tibial marrow. The clumps of cells are adult erythrocytes within dilated blood channels.

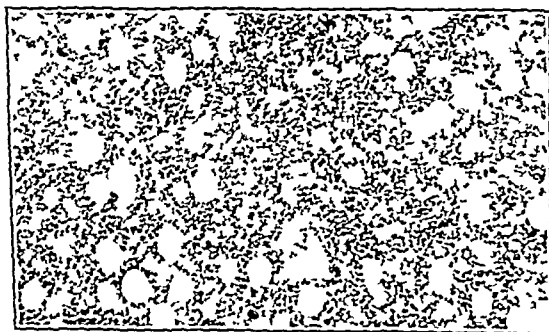


Fig 8

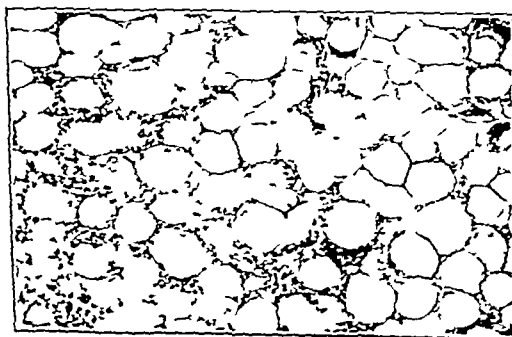


Fig 9

Figs 8 and 9—Two levels of femoral marrow, one centimeter distant from each other, showing wide variation in cellularity.

five to seven millimeters in length with a fine hack-saw and section the bone intact. A thin wedge of a lumbar vertebral body may be removed without difficulty.

Fixation—This step is of great importance and it is imperative to have the tissue removed as soon after death as possible (within eighteen hours the fine cell structures may be lost and the best results can be expected only if the marrow is obtained within eight hours). The fixation of any hemopoietic tissue is most perfect in Zenker-formol solution, composed of nine parts of freshly prepared Zenker's solution (without acetic acid) to which one part of neutral formol (40 per cent formaldehyde over magnesium carbonate) is added within one-half hour after the tissue has been placed in the Zenker's solution. Fixation should extend over four to twelve hours followed by twelve to twenty-four hours washing in running water.

Decalcification—Marrow from the sternum, rib, vertebra, and occasionally from the tibia must be lightly decalcified. For good preservation of cell structure this is best carried out in a mixture of equal parts of an 85 per cent aqueous solution of formic acid and a 20 per cent aqueous solution of sodium citrate. Twelve to twenty-four hours is usually sufficient time to decalcify cancellous bone, the cortex of which has been trimmed away. Decalcification with 5 per cent potassium bichromate gives excellent results but the process often requires several weeks. If this method is used, the solution should not be changed more than once. The tissue must be washed well in running water after decalcification.

Dehydration—The tissue is carried through graded alcohols, beginning with 65 per cent and passing through 80, 90, 95 per cent, absolute (two changes) and equal parts of absolute alcohol and ether. The first two contain iodine to dissolve the mercuric bichloride of the fixative and the last two must stand in a desiccator over anhydrous copper sulphate to insure perfect dehydration.

Embedding—Celloidin is by all means the best, as most of the cell shrinkage is avoided. The method described by Addison in 'Pierce's Normal Histology' is most satisfactory. Paraffin may be used and quite good sections obtained, although cytoplasmic detail is not so well preserved as with celloidin. To cut thin sections with celloidin, an anhydrous technique must be strictly observed in the preparation of the materials, otherwise, the celloidin will be rubbery and impossible to section at less than eight microns thickness.

Staining—A variety of staining methods are available, of which the azur II-eosin is most consistently good.²⁴ Structural detail of nucleus and cytoplasm brought out clearly by this stain is quite lost in preparations stained with hematoxylin-eosin. Details of the method may be found in McClung's "Microscopical Technique."²⁵ A modification of the Ellermann method described by Richter²⁶ gives beautiful results, but it is more difficult and less consistent. The eosin-

*The stains recommended are not applicable to formol-fixed tissue. This may be remedied in part by soaking the cut sections in acetic-free Zenker's solution for about twelve hours. The sections should be washed in running water for several hours and transferred through two weak solutions of iodine in alcohol finally through several changes of 95 per cent alcohol to remove the iodine.

†Sectioning of the tissue should be done at a thickness of 4 microns in paraffin and 5 or 6 in celloidin. The sections must be mounted on albuminized slides from the 1st step and the celloidin removed before staining. Transfer the mounted section through the following solutions: 95 per cent alcohol with iodine (2 changes), 95 per cent alcohol (2 changes), absolute alcohol, absolute alcohol and ether (equal parts), 95 per cent alcohol, 80 per cent alcohol, 60 per cent alcohol, 30 per cent alcohol, water (3 changes), stain.

**Azur II as prepared by the National Aniline and Chemical Company is best.

methylene blue stain is quite good. Sections stained with Wright's blood stain or Pappenheim's modification of the May-Grunewald-Giemsa stain are quite satisfactory.

Mounting—Richter has stated that euparal-mounted preparations are more permanent than those mounted in balsam, the latter having less tendency to fade. We have found neutral balsam quite satisfactory, however, and for ordinary work recommend its use. Gum damar is also good.

Supravital Preparations—The cells of the bone marrow survive for six or seven hours after death and can be studied by the supravital staining technic during this time. A piece of the tissue of about the size of two pinheads is cut with fine curved scissors and carefully transferred to the cover-slip which has been prepared with vital stain according to the technic of Sabin.⁷ The tissue must not be handled with forceps. Apply the slip to a slide with very gentle pressure until the tissue is flattened out. Examination of the main piece allows the tissue between the fat cells to be analyzed, while the free cells along the edges permit of a differential count.

Teased Preparations—Motility of the marrow cells can be observed in teased preparations made soon after death. A tiny bit of marrow from the rib, the size of a pinhead, is squeezed out into a drop of blood serum on a cover-slip and mixed gently. The slip is inverted into the chamber of a hanging-drop slide and the slide transferred promptly to a warm-stage microscope. Cell types are difficult to identify in these preparations. Larger amounts of the marrow may be teased out of the rib, mixed with blood serum menstruum, and smears made in a fashion similar to blood smear preparations. This should be fixed before drying and stained either as a blood smear or as a tissue section.

SUMMARY AND CONCLUSIONS

1 The bone marrow as a tissue presents unusual difficulties for study, particularly due to its delicacy, complexity, inaccessibility, and wide distribution. Its cellular state varies widely in different bones, different levels of the same bone and different areas of a cross-section through a given level.

2 The bone marrow in diseases of the hemolytopoietic system has hitherto been more often haphazardly studied than otherwise.

3 A method is presented, the use of which will result in greater accuracy and consistency in study of the bone marrow.

4 It is hoped that study of the bone marrow by pathologists conducting a routine autopsy service will make available more data relating to diseases of the hemolytopoietic system.

I am indebted to Mr. B. B. Varian of the Department of Anatomy for assistance in developing adequate microscopic technique.

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STUDIES ON THE STRUCTURE AND FUNCTION OF BONE MARROW

II VARIATIONS IN CELLULARITY IN VARIOUS BONES WITH ADVANCING YEARS OF LIFE AND THEIR RELATIVE RESPONSE TO STIMULI*

R P CUSTER, M D , AND FLORENCE E AHLGELDT, M D , PHILADELPHIA, PA

IN A previous paper, attention was called to the fact that study of the bone marrow should not be confined to a sample removed from a single bone. The tibia, femur, rib, sternum and vertebra, as a group, were suggested as preferable bones for marrow study. In the event that such an extensive survey was not considered necessary in an individual case, the femur and vertebra were recommended as the choice bones for examination, the former to determine the response of the blood-forming organs to stimulation, by metamorphosis from fatty to red marrow, the latter to study the cell content.

To establish a rough base line for expectant cellularity at a given age and to determine the relative response of the marrow of the various bones in the face of stimulation, the tibia, femur, rib, sternum and vertebra were observed in a series of one hundred cases, the results being shown graphically on the appended chart. Obviously this can represent only an approximate result, under no circumstances can it be applied with mathematical accuracy to a given case. The number of cases studied in each decade of life are noted in Table I.

TABLE I
AGE DISTRIBUTION BASED ON 83 OF THE CASES

Under 1 month	7
1 month to 1 year	11
1 year to 10 years	9
10 years to 20 years	4
20 years to 30 years	5
30 years to 40 years	9
40 years to 50 years	6
50 years to 60 years	13
60 years to 70 years	12
70 years to 80 years	6
80 years to 90 years	1

CONCLUSIONS

1 Study of the tibia, femur, rib, sternum and vertebra in 100 unselected cases shows that the cellularity of these marrows decreases with advancing years of life, the decrease corresponding in rapidity to the order named. The response

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RELATIVE CELLULARITY OF MARROW OF TIBIA, FEMUR, RIB, STERNUM AND VERTEBRA

A - Normal Variation with Advancing Years of Life (Approximate)

B - Relative Response to Stimuli ("Anger Lungens Induktion")

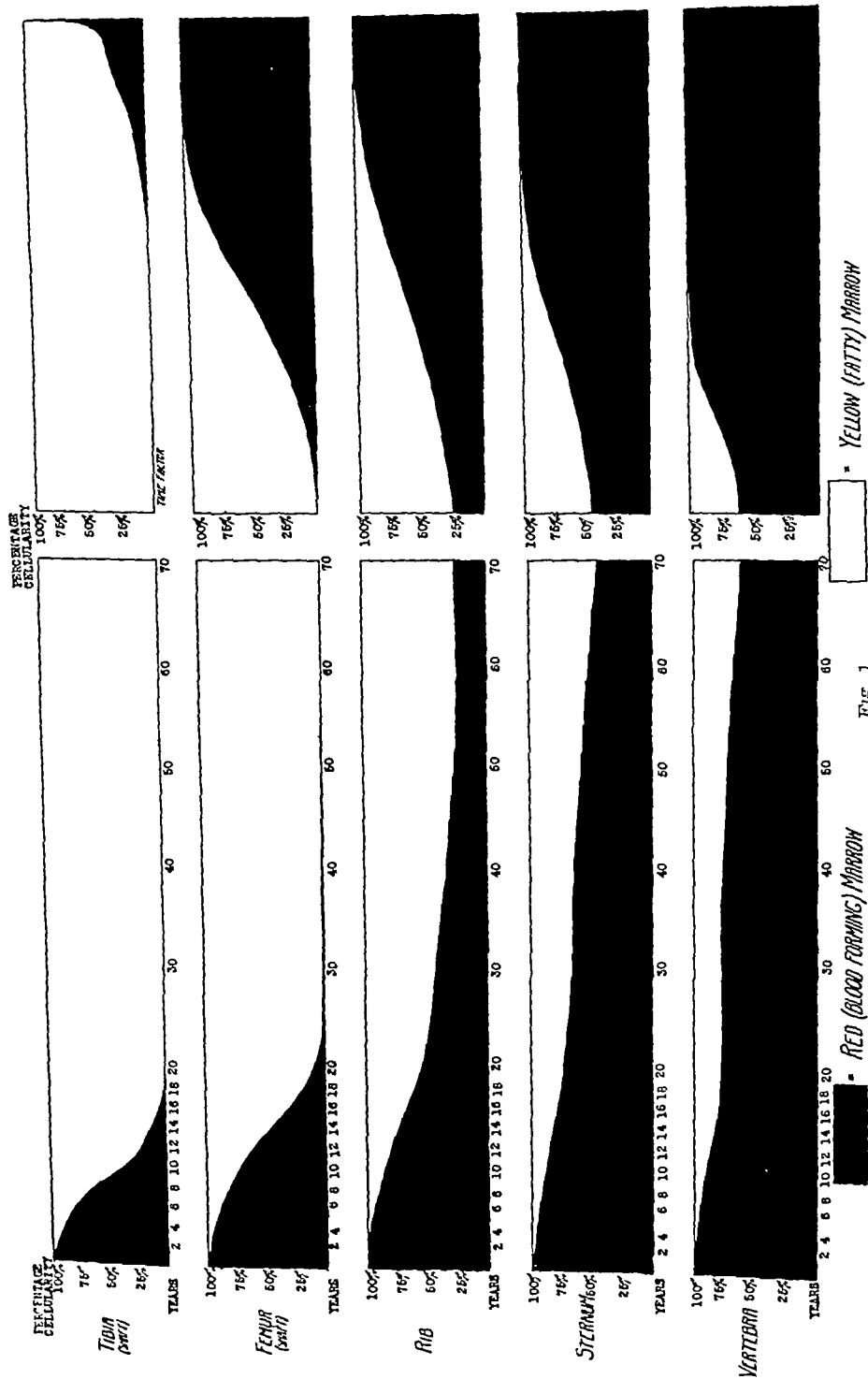


Fig 1

of these marrows to a hemopoietic stimulus of a given intensity is in inverse order, except in the case of the femur, which appears more labile than the rib

2 Bone marrow taken for histologic study at biopsy or necropsy should be selected with these points in mind

3 The previously expressed opinion that the mid-femur is the best single marrow and that femur and vertebra are the best combination for study is confirmed. The sternum is the most suitable bone for biopsy

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Custer, R. P. J. LAB. & CLIN. MED. 17: 951, 1932

A NEUTROPHILIC GRAPH*

ROBERT J. NEEDLES, M.D., DETROIT, MICH.

OUR present knowledge of the blood picture in health and disease is, at best none too complete, it is generously spotted with areas of conjecture. Because of this, any article attempting to deal with the entire field of infection and its effect on the blood would, of necessity, be in the nature of a forecast and not a statement of facts. It is felt, however, that clinicians have not realized the importance of a complete neutrophilic differentiation by one method or another. It is the purpose of this paper to add to the literature a new series of complete neutrophilic differential counts, to give further indication of how such a nuclear partition may be of help in diagnosis and prognosis and to present a new method of graphic representation of the percentage of neutrophils of different age groups as they appear in the differential count.

Since Arneth, in 1904,¹ first demonstrated the clinical significance of the different nuclear forms of the polymorphonuclear neutrophilic leucocytes, the subject has received sporadic attention. Schilling, in 1920,² following the general plan laid down by Arneth, but establishing different criteria for the estimation of hematopoietic activity, evolved a plan which has attained not a little recognition. In 1924 Pons and Krumbhaar³ contributed still another plan of nuclear enumeration, in an attempt to simplify the former methods. Cooke and Ponder, in 1927,⁴ made definite contributions, and Cooke laid down certain criteria for the definite placing of the polymorphonuclear neutrophils in the proper age groups. Finally, in 1930, Farley et al.⁵ presented a new series of normals and contributed another series of pathologic cases, attempting to simplify the methods of previous workers in the field.

TECHNIC

Blood smears are prepared in the ordinary manner, using finger tip or ear blood. It seems immaterial whether slide preparations or cover-slip preparations are used. Either is subject to a certain percentage of error, and neither is of much value unless properly done. The chief essentials are a thin, even smear,

*From the Department of Medicine and the Department of Laboratories, Henry Ford Hospital.

avoiding margination as far as possible. Wright's stain gives quite good results, but for the best differentiation of nuclei, Giemsa's method is preferred. In counting, 100 consecutive white blood cells are classified, as in an ordinary differential, except that as the neutrophils are noted they are placed in the particular age class to which their nuclear lobulation makes them eligible. The time required is but little longer than for the old type of differential count, since it is only necessary to determine the number of true lobules in the polymorphonuclear neutrophilic nucleus to definitely place it. As to the number of cells to be counted, I have given different numbers a rather thorough trial, as well as to check various counts for accuracy, and it seems fair to state that with a good smear, a careful count of 100 cells is sufficient.

Cooke's criterion reads "If there is any band of nuclear material except a fine filament connecting the different parts of a nucleus, that nucleus, for the purposes of the count, cannot be said to be divided." Hence, the neutrophils naturally fall into the five classes as illustrated. Normals for this group have

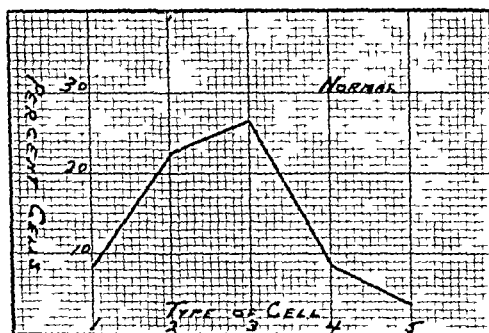


Fig. 1—Total white blood count 7200 polymorphonuclears 70 small lymphocytes 22 large lymphocytes 3 eosinophiles 1.5 basophiles 0.5. This figure is to illustrate the normal neutrophilic partition. Only neutrophils are plotted showing Class 1 8.5 Class 2, 22.5 Class 3 26.7 Class 4 8.5 and Class 5 3.5.

been stated by Cooke and Ponder to be 6.08 per cent of the first, or nonfilament group, while Farley et al found 9.2 per cent. In 100 normal cases, with total counts ranging from 6000 to 8000 mv normals average as follows:

POLY MORPHONUCLEARS					SMALL		LARGE		MONOCYTES	EOSINOPHILES	BASOPHILES
1	2	3	4	5	LYMPHOCYTES	LYMPHOCYTES					
8.45	22.99	26.7	8.7	3.21	20.32	4.32			3.24	1.57	0.5

The above figures are average. It should be understood that there is a normal range varying from 5 to 7 cells per hundred above and below each enumeration. All counts used in compiling these figures were from members of the nursing and service staffs of the hospital. In order to obtain one hundred normal counts it was necessary to examine about one hundred and thirty smears, since it was not unusual to find a nuclear shift in a person who was working eight hours a day and not feeling particularly ill. In most of these abnormal cases a recent cold, a sore throat, or chronic tonsil or sinus disease could be blamed. There were five cases in which no demonstrable reason for the shift could be found at that time.

In this paper, for purposes of clarity, the normal polymorphonuclear neutrophilic percentages are plotted in a broken-line graph, as shown in Fig 1. This normal neutrophilic graph is repeated in all subsequent counts, to indicate the nuclear shift, whether to right or left as Lineth and Schilling have it. Actually, the shift is demonstrated to be clockwise (left) or counterclockwise (right) in these charts. Objection has been raised from time to time in the usage of the

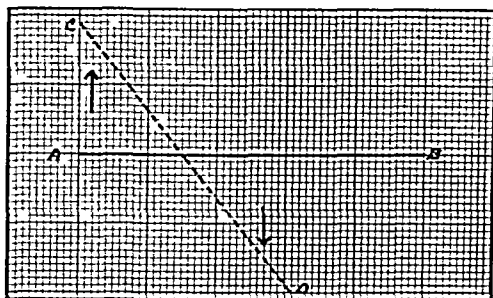


Fig 2—To illustrate the clockwise shift of the nuclear graph with infection. Line AB represents the normal neutrophilic status and CD the relative position of the graph as the number of young neutrophils increases.

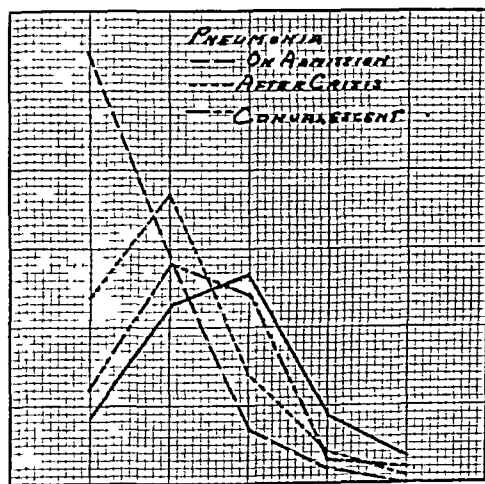


Fig 3—Lobar pneumonia. On admission white blood count 19800 polymorphonuclears 33 small lymphocytes 4 large lymphocytes 3. After the crisis white blood count 12600 polymorphonuclears 80 small lymphocytes 15 large lymphocytes 3 and monocytes 2. Convalescent white blood count 7000 polymorphonuclears 70 small lymphocytes 21 large lymphocytes 7 monocyte 1 and eosinophile 1.

terms "right" or "left" shift. If the picture of the normal neutrophilic graph can be retained, as a balanced beam, with its fulcrum somewhere between the second and third classes, a more striking analogy can be gotten (Fig 2). Thus it will be seen that with an increase in the blood stream of young cells, the balance beam will be thrown clockwise, and at the expense of the cells on the right of the fulcrum, which will therefore move down as the left of the beam moves up, and vice versa. Until a better term is introduced I feel that a clockwise graph best indicates infection, or a neutrophilic shift to the left as interpreted by Schilling.

In the first group of cases (Figs 3, 4, 5, 6, 7), are shown different varieties

of infection, all indicating a more or less pronounced clockwise shift of the nuclear graph, and, with recovery, or subsidence of the infection a counter-clockwise movement back to normal. Thus, in Fig 3 is pictured a case of pneumonia, with graphs drawn to indicate the nuclear shift at the height of the disease, following the crisis, and finally, just before discharge from the hospital. Fig 4 illustrates a case of empyema, with the blood picture approaching normal.

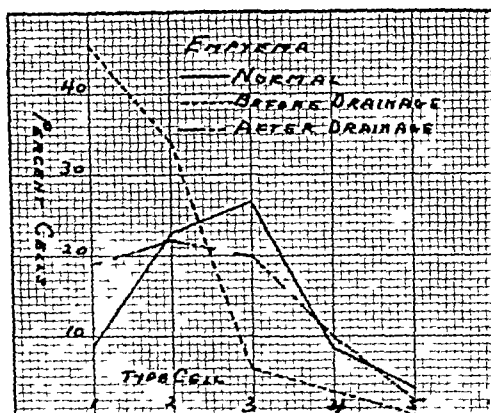


Fig 4—Empyema. Before drainage white blood count 21000 polymorphonuclears 80 small lymphocytes 10 large lymphocytes 2 monocyte 1 eosinophile 1. Drainage was done at once and after two weeks white blood count 8000 polymorphonuclears 74 small lymphocytes 21 large lymphocytes 3 and monocytes 2.

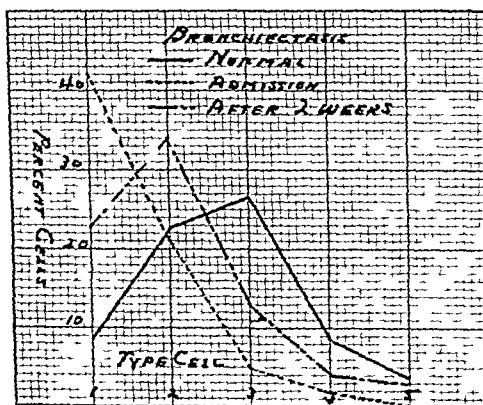


Fig 5—Bronchiectasis with chronic bronchitis in a man fifty four years of age. On admission white blood count 10100 polymorphonuclears 70 small lymphocytes 19 large lymphocytes 6 monocyte 1 eosinophiles 3 basophile 1. After two weeks of postural drainage and inhalations etc white blood count 6800 polymorphonuclears 77 small lymphocytes 10 large lymphocytes 7 monocytes 2 eosinophiles 3 and basophile 1.

uter drainage. Figs 5, 6, and 7 are from relatively milder infections, none of them showing total white blood counts above 8000 yet all before treatment, showing a clockwise movement of the nuclear graph. Evidence seems to indicate, as Farley et al have pointed out and which my own experience seems to confirm, that it is in this field where a careful nuclear differentiation will prove to be of very great help. In these cases presenting a normal total white blood count, and with the normal differential count showing nothing remarkable, it is often possible to show hidden infection by a shift of the nuclear graph. Cases

of chronic tonsillitis, oral sepsis, subacute arthritis (Fig 9) or chronic arthritis (Fig 8) almost uniformly will show a nuclear shift indicative of systemic infection, no matter how chronic or well hidden otherwise

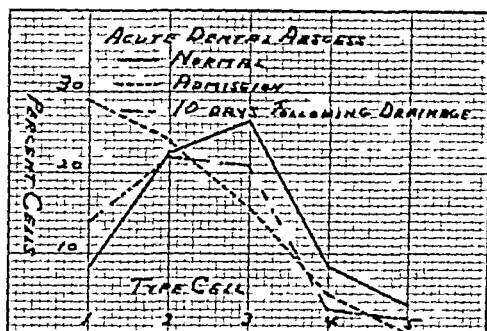


Fig 6—Acute dental abscess On admission white blood count 13200 polymorphonuclears 74 small lymphocytes 16 monocytes 3 and eosinophile 1 Ten days following incision and drainage white blood count 7400 polymorphonuclears 62 small lymphocytes 24 large lymphocytes 7 monocytes 3, eosinophiles 1

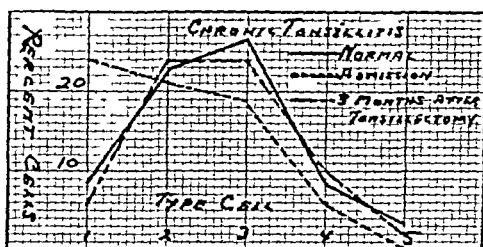


Fig 7—Chronic tonsillitis On admission white blood count 6600 polymorphonuclears 70 small lymphocytes 22 large lymphocytes 4 monocytes 2 eosinophile 1 basophile 1 Tonsillectomy was done and the patient discharged from the hospital after recovery Three months later on returning to the hospital for check-up white blood count 7000 polymorphonuclears 66 small lymphocytes 26 large lymphocytes 2 monocytes 3 eosinophiles 2 basophile 1

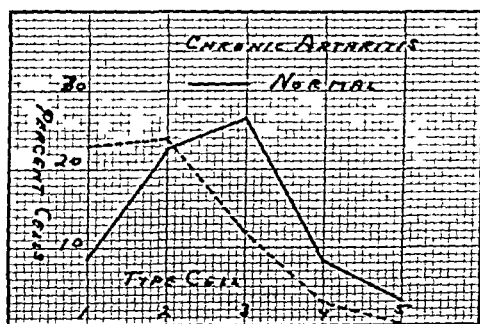


Fig 8—Chronic arthritis In this patient all possible foci had been either checked and found negative or else cleaned out but the neutrophilic graph shows a distinct clockwise shift. Her symptoms at this admission were localized to one ankle and the shift points to this as the infectious basis for neutrophilic stimulation White blood count 7000 polymorphonuclears 62 small lymphocytes 30 large lymphocytes 2 monocytes 4 eosinophile 1 and basophile 1

Obscure abdominal conditions, such as are pictured in Figs 10 and 11, may well be mistaken for the more common acute intraabdominal lesions In Fig 10 is shown the nuclear curve from a case of ovarian carcinoma, which was producing confusing symptoms In Fig 11 is an ectopic pregnancy which could easily

have been mistaken for an acute salpingitis or appendicitis. In both cases, the absence of a marked nuclear shift was against an acute suppurative condition.

Patients presenting vague symptoms, those in whom the question always

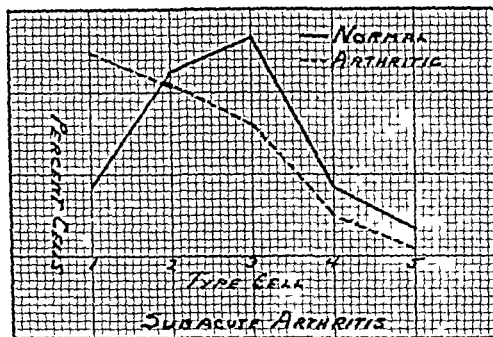


Fig. 9—Subacute arthritis. Patient showed residual symptoms in one knee and in lumbar spine. White blood count 7500 polymorphonuclears 68 small lymphocytes 21 large lymphocytes 4 monocytes 4 and eosinophiles 2, and basophile 1.

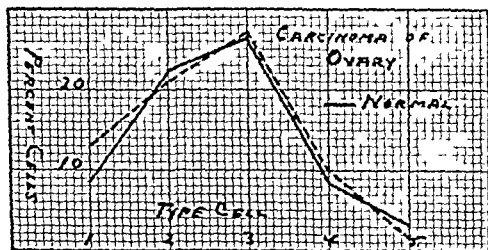


Fig. 10—Carcinoma of ovary. White blood count 7600 polymorphonuclears 77 small lymphocytes 18 large lymphocytes 2 monocytes 4 and eosinophiles 3.

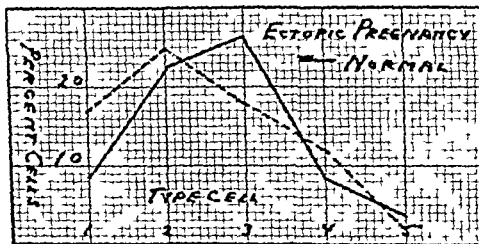


Fig. 11—Ectopic pregnancy. Patient was a young white woman twenty-four years of age whose story and symptoms might have been either an acute appendicitis or in acute salpingitis. The neutrophilic graph shows her nuclear partition to be very little above normal. White blood count 13800 polymorphonuclears 74 small lymphocytes 17 large lymphocytes 3, monocytes 4 eosinophils 1 basophile 1.

arises as to whether the picture is functional or organic, offer an interesting field for the nuclear graph. In Figs 12, 13, 14, and 15 are shown cases which seem to be of functional etiology. None of them offer any nuclear shift to indicate infection, and some support for the functional diagnosis is thereby gained.

That the blood-forming tissues do anticipate demonstrable signs of infection, and begin early, to indicate activity in relation to such an invasion is shown in Figs 16 and 17. In Fig 16 the neutrophilic graphs from three persons given the first immunizing dose of toxin antitoxin. Of the three, two showed slight rises

in total white blood cells, the third a leucopenia of 4200, and yet all three showed a distinct clockwise shift in the nuclear graph. In Fig 17 are the nuclear curves of three women in the last week of pregnancy. All were afebrile and as far as a careful clinical examination showed, none of the three had any infection present. The total white blood counts ranged from 8200 to 9600. A nuclear shift in advance of any other sign of infection is thus demonstrated. It seems probable that this phenomenon takes place in many conditions, where the neutrophile

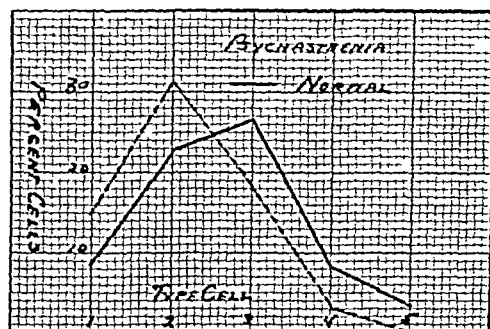


Fig 12—Psychasthenia showing practically a normal nuclear partition. White blood count 7800 polymorphonuclears 67 small lymphocytes 28 large lymphocyte 1 monocytes 3, eosinophilic 1.

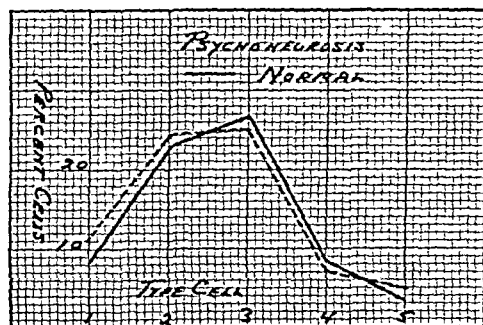


Fig 13—Psychoneurosis. This patient presented vague complaints many of them and the nuclear partition of the neutrophils as pictured above points to a functional basis for his trouble. White blood count 7600 polymorphonuclears 72 small lymphocytes 18 large lymphocytes 5 monocytes 3 eosinophile 1 and basophile 1.

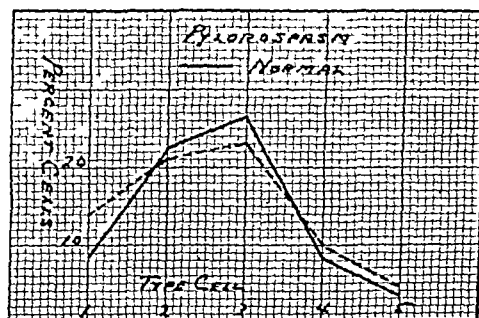


Fig 14—Pylorospasm. Differentiation here lay between a chronic peptic ulcer with obstruction or nervous pylorospasm. The neutrophilic partition points to a functional basis. White blood count 7700 polymorphonuclears 73, small lymphocytes 22 large lymphocytes 2 monocyte 1 eosinophile 1 and basophile 1.

cells increase to take care of an infection, real or potential, without clinical manifestations, other than might be shown by a neutrophilic count

In Fig 18 are pictured three cases of uncomplicated arterial hypertension. Of six cases available, these three were selected because no possible source of infection could be located. All three cases, however, with normal total white blood counts, showed a nuclear shift indicative of low grade infection. I do not wish to be placed in the position of attributing hypertension to chronic infection, but

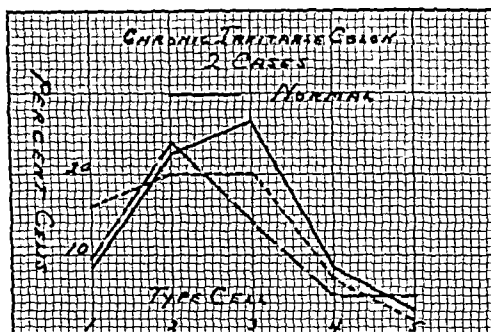


Fig 15—Chronic irritable colon (spastic colitis). Neither case shows a white blood count above 7400 and neither shows much evidence of infection in the neutrophilic nuclear partition

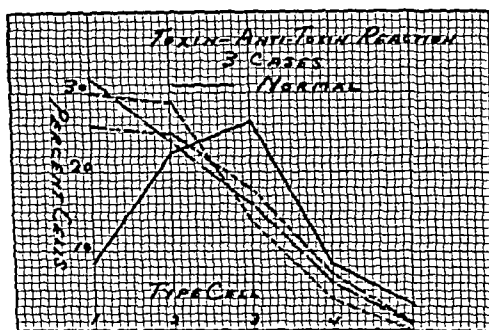


Fig. 16—Toxin antitoxin reaction in three student nurses. None of the three developed any serious sequelae. The neutrophilic partition however points to a distinct neutropoietic stimulation

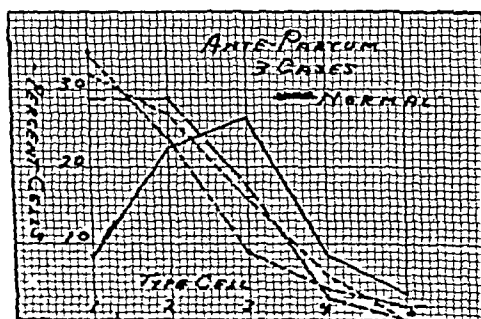


Fig. 17—From three women in the last week of pregnancy. None of the three had any clinical sign of infection at this time and all three passed through labor delivery and two weeks postpartum in the hospital without unusual symptoms

merely point out that in three cases of hypertension, where infection could not be demonstrated, a nuclear curve was obtained which is probably indicative of infection

Infectious mononucleosis is ordinarily interpreted as a disease process which stimulates primarily the lymphocytic centers, with a consequent increase in the large and small lymphocytes in the blood stream Fig 19 is presented merely to

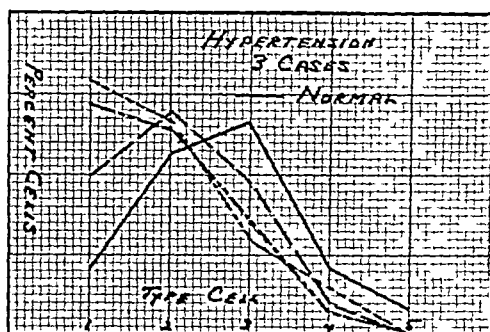


Fig 18—Hypertension three cases Total white blood count ranged from 7000 to 7400 No clinical signs of infection and no demonstrable foci

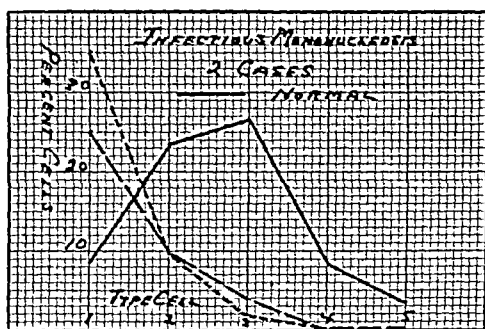


Fig 19—Infectious mononucleosis two cases Case 1 white blood count 4600 polymorphonuclears 39 small lymphocytes 17 large lymphocytes 41 and monocytes 3 Case 2 white blood count 11200 polymorphonuclears 47 small lymphocytes 15 large lymphocytes 32 and monocytes 6

show that, even with but 20 to 40 per cent of polymorphonuclears in the stained smear, a strong nuclear shift is demonstrable The mechanism is not clear, except that this is also an infectious condition, nor is the fact of a relative neutropenia in the face of marked neutropoietic activity discernible at this time

Interesting phenomena occur in Figs 20 and 21 In the former a case of ulcerative colitis is shown, where, week after week, a steady nuclear shift occurs, with few, if any, of the last or older three groups of polymorphonuclears being seen As is shown, however, immediately after a transfusion, the old forms may be picked up in the stained smear, in small percentage These are probably from donor's blood In Fig 21 is a case of hemorrhaging peptic ulcer, with a nuclear graph showing the status before and after transfusion, and also after development of a terminal bilateral suppurative parotitis Although this case had carried a leucopenia of from 3000 to 5000 during twenty days in the hos-

pital, in spite of a nuclear shift as shown, on the development of the suppurative process the white blood cells shot to 18000 and the clockwise shift tremendously increased

In Fig 22 is shown a fatal pneumonia, with a relative leucopenia (9600) As indicated, the clockwise shift was profound, and, while it cannot be said that such a graph is indicative of overwhelming infection, it is nevertheless probable

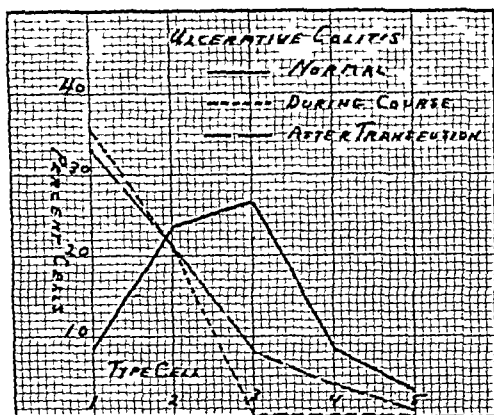


Fig 20—Ulcerative colitis First count taken two hours before transfusion is sample of many taken during long hospitalization Total white blood counts ranged from 8000 to 18000 First count white blood count 12200 polymorphonuclears 67 small lymphocytes 24 large lymphocytes 4 monocytes 3 eosinophiles 2 Two hours after being given 100 cubic centimeters of citrated blood the count was white blood count 12600 polymorphonuclears 67 small lymphocytes 21 large lymphocytes 4 monocytes 6 eosinophiles 2

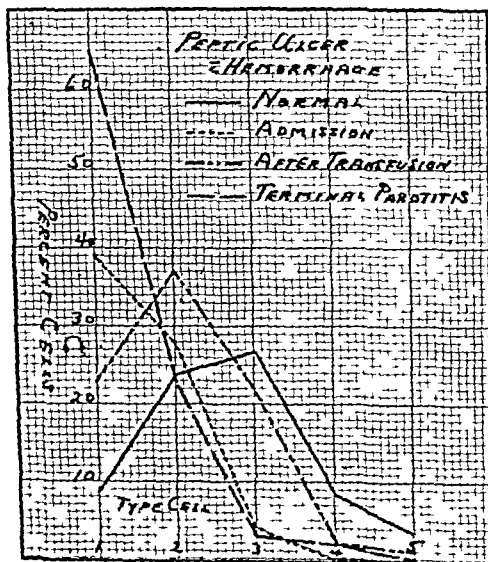


Fig. 21—Peptic ulcer with repeated hemorrhages Count taken two hours before transfusion shows white blood count 4200 polymorphonuclears 71 small lymphocytes 20 large lymphocytes 1 eosinophils 1 Two hours following transfusion of 400 cubic centimeters citrated blood the count was white blood count 3000 polymorphonuclears 83 small lymphocytes 14 large lymphocytes 2 and monocyte 1 Six days later the patient developed a bilateral suppurative parotitis and at this time the count was white blood count 18000 polymorphonuclears 93 small lymphocytes 1 large lymphocytes 2

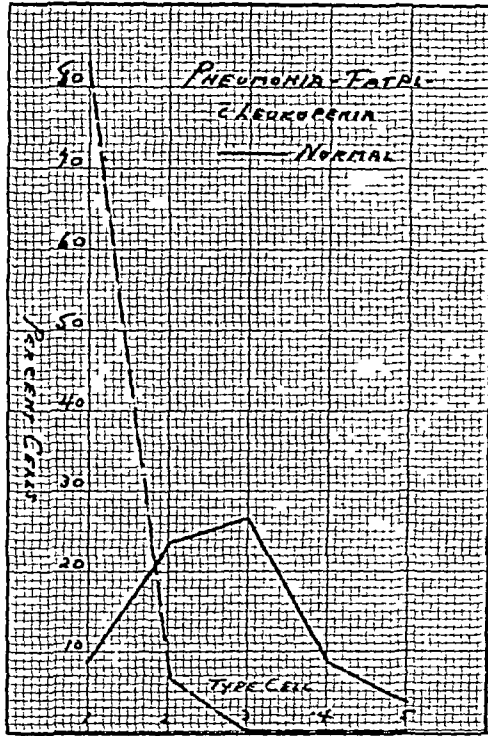


Fig. 22—Type I pneumonia in a man of sixty years. Admitted to hospital on fourth day of illness. White blood count 9600 polymorphonuclears 90 small lymphocytes 6 large lymphocytes 4. Patient died twelve hours later.

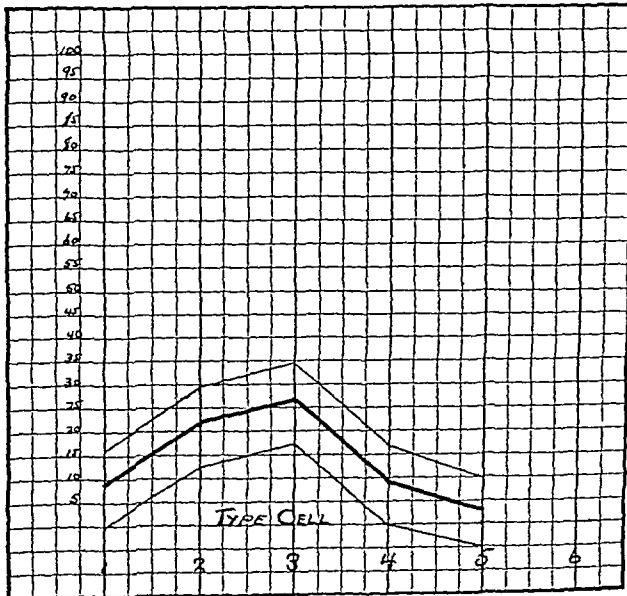


Fig. 23—Suggested form for routine plotting of neutrophilic partition.

that a shift to such a degree is to be expected in the more profound and grave types of infection

Fig 23 shows a form of chart which may be adapted to clinical use. On it are shown, in heavy black line, the average normal figures, while above and below are lighter lines which might be said to be the upper and lower limits of normal in each cell group.

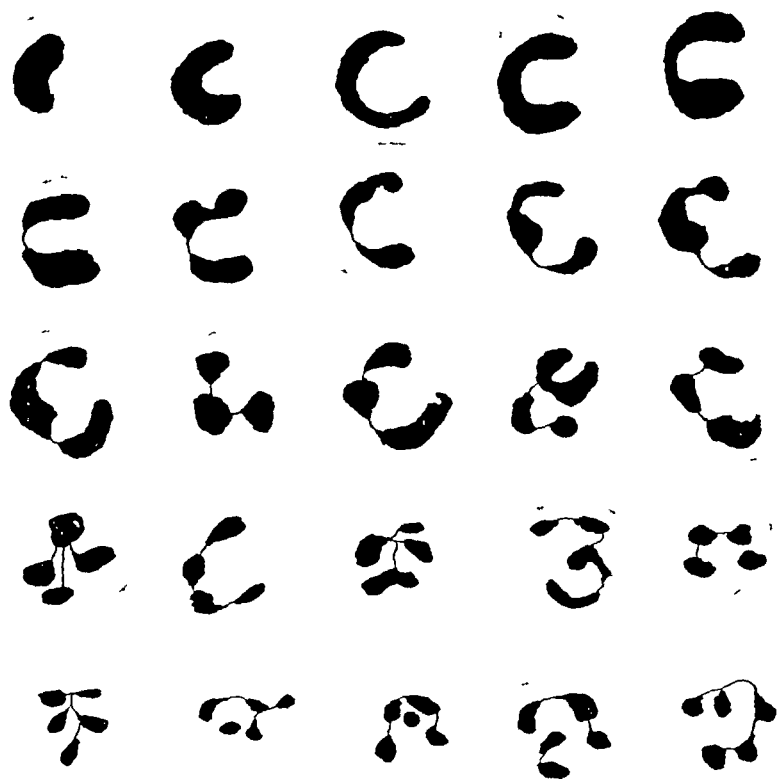


Fig 24

SUMMARY

- 1 Recognition is given to pioneers in the field of differential neutrophilic counts
- 2 A new series of differential neutrophilic counts is presented, with average normal figures for each of the common cell-types in the stained smear
- 3 A new method of graphically presenting the nuclear shift is proposed
- 4 Graphs are shown from various conditions in support of the thesis as cited
- 5 It must be stated again that this paper is not an attempt to cover the field. The way is only just opened. It is hoped that in future papers the blood picture in various diseases will be more thoroughly investigated in the light of present day knowledge as to the importance of the complete neutrophilic differential count and its graphic illustration

CONCLUSIONS

1 The normal number of nonfilament or one lobed polymorphonuclear neutrophilic leucocytes in the blood stream is from 6.08 to 9.2 per cent and in the series presented, 8.45 per cent. These figures are average.

2 The normal number of the older neutrophiles is determined to be as follows

2 lobes—	22.99%
3 lobes—	26.7%
4 lobes—	8.7%
5 lobes—	3.21%

3 Infectious processes will cause a definite shift in the neutrophiles the extent depending on the severity of the infection.

4 This shift takes place whether with or without a rise in the total number of circulating white blood cells.

5 There is reason to believe that hidden or focal infection can be demonstrated by this method.

6 There is also reason to believe that acute purulent conditions may be better differentiated from other conditions causing like symptoms.

7 Evidence is presented to show that functional conditions may be better differentiated from organic conditions.

Acknowledgment is made for valuable advice and cooperation in the preparation of this paper to Dr. F. J. Sladen and Dr. F. W. Hartman.

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THE AGE OF THE LEUCOCYTE IN RELATION TO INFECTION^{*}

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THE leucocytes, or white cells of normal blood are classified as neutrophiles, eosinophiles, basophiles, monocytes, and lymphocytes. The changes in absolute and relative numbers of these various groups of cells, as a result of infection or other disturbance, are not within the scope of this review. Such numerical changes constitute the ordinary data of absolute and relative leucocytosis and leucopenia, which have received the major share of attention in clinical hematology for many years.

Study of correlated qualitative changes within the members of the several white cell groups dates back to Ehrlich, but has not found widespread clinical application until recent times.

Arneith's¹ pioneer researches (1904) constitute the ground-work and at the same time mark the point of departure for subsequent developments. Arneith's hypothesis in regard to the neutrophile's response to infection is based on two correlated major premises: (1) that young neutrophiles are supplied in abnormally increased numbers to the blood stream as a fundamental defense mechanism against infection, and (2) that the age of neutrophiles varies directly with, and can be measured in terms of, nuclear polymorphism or lobulation. Arneith extended his general theory to include other white cells,² but the major interest has centered about the neutrophiles.

THE MEANING OF "YOUTH" AND "MATURITY" OF LEUCOCYTES

When hematologists designate a given circulating leucocyte as "young" or "mature" or "senile" there are a number of conditional assumptions implied, and the meaning of such age judgments may vary considerably. A statement of "age" presupposes knowledge of a beginning. It seems fair to suggest that, when dealing with circulating leucocytes, the "beginning" is thought of vaguely in terms of either moment of entry into the blood stream, or moment of definitive differentiation in the formative center, or a combination of both. Thus a myelocyte in the peripheral blood is a "young" form because, first, it is assumed that a living myelocyte quickly matures into a neutrophile leucocyte after liberation from the formative tissue, and second, its morphology identifies it with the early granule containing cell in the formative tissue. Neither of these assumptions, however, need necessarily be valid.

A myelocyte in the blood stream might conceivably be a relatively stable and long lived individual analogous to a "dormant" cancer cell or a bacterial spore. If this should be true its presence in the blood stream would lose all significance, for purposes of age judgments, from this standpoint. Viewed from another angle, the "aging process" may produce varying definitive stages with different "spins" of life under varying conditions. No one knows how long

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a given myelocyte may remain undifferentiated, growing "older by the clock," before it "matures" or "divides" or before it enters the blood stream. No one knows with certainty the "normal life-span" of the neutrophile in the blood. It is estimated in terms of "hours" by some,³ "days" by others⁴ and "weeks" by others.⁵

In spite of these theoretical uncertainties, the working concept of "youth" and "age" judgments of leucocytes is that *a leucocyte is young in all senses of the word when it looks and behaves like its embryologically established (or assumed) progenitor in the leucopoietic centers*.

GENERAL CRITERIA OF YOUTH OF LEUCOCYTES

The morphologic evidences of immaturity of leucocytes may be conveniently considered under (a) nuclear and (b) cytoplasmic

NUCLEAR EVIDENCES OF IMMATURITY

1 Mitosis and amitosis are universally admitted as certain proof of immaturity. This is especially true of mitosis, which cannot be simulated, by cell rupture or other artifact, as easily as amitosis. Both forms of cell division have been observed in practically all of the immature leucocytes in the formative centers, as well as in the blood stream in leucemias. Maximow⁶ insists that amitosis does not occur in lymphocytic cells, but other observers disagree.⁷ In the "granular series" cell division is demonstrable in the myeloblast, the promyelocyte and the myelocyte but not thereafter (i. e. not in metamyelocytes "band forms" or polymorphonuclear neutrophiles). In the lymphatic series Maximow (loc. cit. p. 329) describes mitotic figures in large and in medium sized lymphocytes and states that small lymphocytes do not divide. Sabin emphasizes amitosis as the usual form of myeloblastic division.

In clinical hematology (except in study of the leucemias) *the occurrence of peripheral leucocytic mitosis and amitosis is of little practical value in estimating age for the reason that almost all circulating leucocytes are relatively "mature" and are incapable of further cell-division*.

2 Nucleoli within the nucleus of leucocytes constitute valid proof of immaturity. The actual demonstration of nucleoli is, however, not always an easy and certain procedure. In the granular series the myeloblast nucleus is, according to all observers, characterized by the presence of two to six nucleoli. The same is true of the promyelocyte. The myelocyte nucleus, however, presents uncertainty. Downey⁸ states that the myelocyte proper and its descendants (metamyelocytes, polymorphonuclear neutrophiles, eosinophiles and basophiles) are totally devoid of nucleoli. Bunting (loc. cit. p. 405) and Schilling,⁹ on the other hand, find nucleoli in myelocytes and metamyelocytes, and Naegeli¹⁰ agrees that occasionally nucleoli may be seen in myelocytes. All authorities concur in the opinion that ordinary polymorphonuclear neutrophiles have no demonstrable nucleoli. Bunting finds, however (loc. cit. p. 405) that special staining may bring out nucleoli in the young polymorphonuclear neutrophile, eosinophile, and basophile cells of the blood.

In the lymphocyte series the lymphoblast nucleus is unanimously accorded nucleoli, although authorities differ as to the characteristic numbers (1 to 2

nucleoli according to Naegeli and Schilling, up to 5 or 6 according to Schridde and Butterfield) The large "young" lymphocyte of the blood may have nucleoli (Schilling, loc cit p 131) but mature lymphocytes have none (Downey, loc cit p 375)

The monoblast (Naegeli) may contain nucleoli Concerning monocytes proper there is some disagreement, no nucleoli according to Downey (p 376), occasionally present according to Schilling (p 132) and Naegeli (p 143) who emphasizes the importance of vital stains in this connection

From this summary it is clear that the *status of nucleoli in the several leucocyte types is hardly definite enough to offer much help in clinical hematology except in research work and in the study of leucemias*

3 Chromatin structure of the nucleus is recognized, in stained preparations, as a most significant criterion of age Young leucocytes possess vesicular nuclei formed like a net built of fine basic staining threads (Naegeli p 178) The nucleus of older leucocytes is more coarsely constructed with heavier bands and pieces of deeper staining basic chromatin Older nuclei also show either a tendency to pyknosis or a tendency to sharp differentiation of basic and oxychromatin, which are features lacking in young nuclei The nuclear membrane is thin and indistinct in very young cells and is thicker and clearer in older cells, with a submembranous condensation of basic chromatin that emphasizes it all the more

The chromatin structure of the nucleus of leucocytes is considered by many hematologists to be the soundest and most useful general criterion of age

4 Nuclear polymorphism or lobulation as an index of leucocyte maturity constitutes the major premise of Aineeth's theory All observers are agreed that the nuclei of primitive white cells are round or oval and that the nuclei of most mature leucocytes (lymphocytes excepted) show more or less indentation, lobulation, and polymorphism Aineeth holds, for the neutrophiles particularly and for other leucocytes to a certain extent, that age duration and multiplicity of nuclear lobulations are directly and mathematically correlated

This fundamental position of Aineeth's is attacked by certain other hematologists along several lines For example Naegeli says (loc cit p 178) "entirely erroneous is the procedure of Aineeth by which the age of the cell is estimated from the degree of segmentation of its nucleus There are many cells most certainly young as judged by nuclear structure which are markedly segmented, especially is this true of monocytes but also of neutrophiles" Bunting says (loc cit p 405) "leucocytes with basophilic protoplasm, loosely woven nuclei and even with basophilic granules among the neutrophilic granules and thus obviously young cells may show as many lobes to the nucleus as cells evidently senile"

The practical application and developments of Aineeth's doctrines will be discussed below, together with their difficulties and limitations The above remarks indicate the somewhat *uncertain status of the basic evidence of Aineeth's contention*

CYTOPLASMIC EVIDENCES OF IMMATUREITY

1 Mitochondria demonstrable by supravital stains (e g Janus green), are present in all young white cells These structures seem to diminish in number

as the cell matures Sabin¹ finds that, as the myeloblast grows older, the multitudinous mitochondria (staining green in the neutral red Janus green mixture) become fewer and the specific (red staining) granules begin to make their appearance. In the mature myelocyte there are very few mitochondria and more "specific" granules, and in the mature polymorphonuclear neutrophile there are no mitochondria at all. Similarly according to Sabin and her coworkers, the monoblast with many (green staining) mitochondria matures into the large mononuclear type of monocyte with fewer mitochondria and this in turn into the "transitional" type of monocyte with but very few mitochondria (and a fully developed neutral red rosette). A similar diminution of mitochondria is described in the transition of the lymphoblast to the lymphocyte.

The practical uses of "supravital" stains in clinical hematology have been directed more toward attempts at differentiation of cell types than toward estimation of cell maturity. *It would seem that a careful study of leucocytes by supravital technic correlated with the ordinary dry smear stains (Wright's, etc.) in each instance might yield valuable evidence regarding the validity of our ordinary morphologic criteria of youth and maturity.*

2 Cytoplasmic basophilia is a generally accepted indication of relative immaturity of circulating leucocytes.

3 The centrosome and the Golgi apparatus, when present, are probably evidences of relative immaturity. The demonstration by special staining methods of these structures has, as yet, no place in clinical hematology.

4 Cell size is of little or no value as a criterion of age except in this very general way: the progenitors of the blood leucocytes tend to become smaller as they mature (Naegeli p 179). Young neutrophiles are said to be larger than mature ones (Schilling p 128) but the "macropolycyte" of Cooke and the large "pernicious anemia neutrophile" would seem to be obvious exceptions to this general statement. In regard to lymphocytes there is also no established correlation between cell size and age. The large lymphocytes are not necessarily younger than the small and medium sized lymphocytes (Naegeli p 135, Maximow p 329, 358). Finally the stem cells themselves are found to present marked variation in size in the formative centers and their descendants in the blood stream are subjected not only to these inherent developmental variables but also to extraneous factors such as osmosis and hydropic changes, all of which must have an influence on "size."

5 Cytoplasmic granulation (as it appears in polychrome stains) in the neutrophile series of leucocytes presents features of importance in relation to the age of these cells. Young neutrophiles have more basic staining granules scattered in with the neutrophilic granules than do the mature forms. In the latter the specific granulation of the cytoplasm is purely neutrophilic or even slightly acidophilic in quality.

This tendency to basic granulation of young neutrophiles is recognized as a most significant criterion of immaturity by most observers.

6 Amoeboid activity and phagocytic activity of circulating leucocytes in warm-stage preparations are acceptable evidence of vitality and functional efficiency but have no direct bearing on the problem of age. Obviously dead and

*A correlation study of this sort is being planned in this clinic in collaboration with Byron E. Hall.

dying cells will not exhibit these phenomena but not all dead cells are old cells, especially in toxic and diseased stages. Furthermore young leucocytes are not necessarily more active than older cells of the same type. It is recognized, for example, that myeloblasts and myelocytes show much less evidence of these sorts of activity than do mature neutrophiles.

The Age of Leucocytes (Other Than Neutrophiles) in Relation to Infection—As previously indicated the chief interest in the age of leucocytes in relation to infection centers around the neutrophile. Arneth has attempted to bring the other white cells into line with his theories but this attempt has met with little or no recognition of success (see Schilling, loc cit p 147). We have reviewed briefly the general criteria that are applicable. The difficulties are obvious.

These difficulties become still greater when one digs deeper into the mass of data at hand. "In cases of monocytosis, transitions between lymphocytes and monocytes in the blood are fairly numerous" (Maximow loc cit p 463). Small lymphocytes do not divide but "may change over into large lymphocytes or monocytes or plasma cells" (Maximow, p 329-349, 358). Plasma cells are identical with Turk irritation cells and are thought to arise from lymphocytes by Maximow, from myeloblasts by Downey (loc cit p 377) and from both types of stem cell by Naegeli (loc cit p 176-177). The so-called Rieder cell is a circulating myeloblast with indented nucleus according to Downey, who admits the great difficulty of differentiating it from monocytes. The "Rieder type" is of lymphoblastic origin according to Schilling (p 135). This observer notes the occasional "incongruity in the maturity of nucleus and protoplasm" in various types of leucocytes. We have already indicated the impropriety of correlating the large and small lymphocyte with the young and old lymphocyte. Concerning eosinophilic leucocytes there is little or nothing of practical value that is known in this connection. We have personally studied the blood of a patient with eosinophilic hyperleucocytosis¹³ in which the striking feature was the presence of huge numbers of perfectly normal looking "adult" eosinophiles with polymorphous nuclei and no eosinophilic myelocytes or other evidence of "shift to the left" of the eosinophile formula, although at necropsy this patient's bone marrow was found packed with eosinophilic myelocytes. We have on the other hand seen mononuclear eosinophiles (young forms?) in the blood of a case of trichiniasis which recovered.¹⁴

The Age of the Neutrophile in Relation to Infection—The Arneth "count" of neutrophile nuclear lobulations, and to a certain extent the modifications of this procedure (the Schilling Hemogram, the polynuclear count of Cooke and Ponder, the nonfilament neutrophile count of Farley, etc.) are open to criticism and objection from several standpoints (1) embryologic, morphologic, and physiologic, and (2) technical and utilitarian.

Before considering the subject from these angles, a brief review is necessary of the theory and practice of Arneth and his successors.

The Arneth Count—The neutrophile cells of normal blood, according to Arneth are classifiable into five groups. Class I (5 per cent of neutrophiles) are cells whose nucleus is round, oval, or indented but not truly lobulated. Class II (35 per cent of neutrophiles) are cells with two lobed nuclei. Class III (41 per cent of neutrophiles) are cells with three lobed nuclei. Class IV (17 per

cent) are neutrophiles with four lobed nuclei, and Class V (2 per cent) are neutrophiles with five lobed nuclei. Aineeth makes numerous subdivisions within each class so that in all he recognizes twenty distinct types of circulating neutrophiles. He believes that increased nuclear lobulation is direct evidence of increased age from beginning to end. He further believes as a direct result of his laborious researches, that a "shift to the left" in the neutrophile formula occurs as a result of infection. By this he means a relative increase in the "young" neutrophiles belonging in the "left hand" columns of cells (Classes I and II). According to Aineeth a proper study of the "nuclear shift" constitutes a more sensitive index of the presence of and trend of infection than does the ordinary "total leucocyte and differential count" usually employed.

As to the mechanism of leucocytosis and shift to the left in infection Aineeth contends that this represents a disturbance of the normal balance between peripheral leucocyte destruction and central (secondary) new cell production.

The Schilling Hemogram—Victor Schilling recognizing the merit of Aineeth's (overcomplex) procedure, made certain important modifications. He felt that Aineeth had gone too far in correlating increasing maturity with increasing nuclear lobulation. Schilling therefore grouped all the cells of Aineeth's Classes II to V inclusive into one class as "segment-nuclear" (mature) neutrophiles (63 per cent of the normal total leucocyte count, 94 per cent of the normal neutrophiles). The other neutrophilic granular cells of the blood are classified by Schilling as myelocytes (absent in normal blood), "rod nucleais" or "band forms" corresponding to mature metamyelocytes (4 per cent of the normal total leucocyte count, 6 per cent of the normal neutrophiles), "young" or "juvenile" neutrophiles, corresponding to Pappenheim's young metamyelocytes and differing from the "rod nucleais" chiefly by the sausage or bean-shaped character of the nucleus with nucleoli in contrast to the rod-shaped nucleus without nucleoli, (these juvenile forms are designated by Schilling as "rarely occurring" in normal blood but sometimes present in numbers up to 1 per cent of the total leucocytes), a special degenerative "stab" or staff form of nonsegment nuclear neutrophile which is according to Schilling a "neutrophile matured without segmentation." These "degenerated rod nucleais" or "stab" forms are absent from normal blood and are of especial significance in Schilling's theory and practice, in that they represent a distinct contradiction of Aineeth's fundamental theory, and play a major rôle in Schilling's hypothesis of a "degenerative shift to the left."

Schilling's hemogram includes a tabulation of (1) myelocytes, (2) juvenile neutrophiles (young metamyelocytes), (3) "rod-nucleais" or "band forms" (old metamyelocytes) (4) "stab" nucleais (a degenerative nonsegment nuclear of special type), and (5) segment nuclear ("adult") neutrophiles together with (6) the usual differential count of lymphocytes, monocytes and eosinophiles. Each of these items in the hemogram is expressed in percentage of total leucocytes (and not, as Aineeth had done in percentage of neutrophiles).

Cooke's Polynuclear Count—Aineeth's criteria for separating the number of nuclear lobes were not specific. Schilling gave more attention to this difficulty, but since he grouped all neutrophiles with more than one lobe into one class (of "segment-nucleais"), he was not greatly concerned about the question. Cooke

(1914) regrouped the "polymorphs" into five classes analogous to Arneith's classes, but more strictly defined" by the following morphologic dictum "If there is any band of nuclear tissue except a chromatin filament connecting the different parts of a nucleus, that nucleus cannot for the purposes of the count, be said to be divided"

On this basis Cooke's Classes III, IV, and V are practically identical with Arneith's Classes III, IV, and V Cooke's Class I however, for some curious reason does not include the myelocyte or the young metamyelocyte It includes all of the cells of Arneith's Class I except myelocytes and metamyelocytes and some of Arneith's Class II (i e those with twisted nuclei showing a central construction but not a true "filament") Cooke's Class I includes all of Schilling's nonsegment neutrophils (except the myelocyte and young metamyelocyte) Cooke's Class II is identical with Arneith's Class II, except for a few nonfilament nucleals with "pseudo bilobulation" which Cooke's criterion makes him classify as "undivided" (i e as belonging in Class I) In normal blood the average number of Class I cells is 10 per cent of the neutrophils (or about 7 per cent of the total leucocytes) Cooke's findings and views concerning the "macropolyocyte" are outside the scope of this review, except that they constitute a partial break in his otherwise whole hearted allegiance to Arneith's general hypothesis

"*The Simplified Arneith Count*" of Pons and Krumbhaar —Pons and Krumbhaar (1924) adopted Cooke's criterion of the "filament" but suggested¹⁰ that the essential feature of the neutrophil count could be maintained by a three fold classification into (1) metamyelocytes, (2) nonsegmented band forms, and (3) segmented forms with filament connections between the lobes

The Filament and Nonfilament Polymorphonuclear Neutrophil Count —Fairley¹¹ and his coworkers (1930) using Cooke's criterion of the filament, divide the neutrophils into nonfilament forms (practically identical with Cooke's Class I) and filament forms (Cooke's Classes II to V inclusive) and report them separately as components in the ordinary complete differential count These workers report an average of 9 per cent nonfilament neutrophils in a study of 100 normal individuals and set 16 per cent as the upper limit of normal These figures represent percentage of total leucocytes (not percentage of neutrophils)

Our own procedure (since 1928) also based on Cooke's criterion, has been to include in the routine differential count, a bracket after the figure for the polymorphonuclear neutrophil percentage, into which are set down the percentages of "old" and "young" forms seen in making the differential count of 100 white cells A "normal differential" would be as follows Polymorphonuclear neutrophils 62 per cent (56 per cent "old", 6 per cent "young"), lymphocytes 29 per cent, monocytes 6 per cent, eosinophiles 3 per cent, basophiles 0, myelocytes 0 A "typical" count in very severe infection would be Polymorphonuclear neutrophils 88 per cent (45 per cent "old", 43 per cent "young"), lymphocytes 9 per cent, monocytes 2 per cent, eosinophiles 0, basophiles 0, myelocytes 1 per cent

In our procedure the designation of "young" forms comprises all nonfilament neutrophils (i e all of Cooke's Class I cells) and all metamyelocytes as well (i e all of Schilling's "juvenile forms" "rod-nuclears," and "stab"

forms) Farley's classification is identical and serves the added good purpose of employing a more exact descriptive terminology (i e filament and nonfilament instead of "young" and "old")

Certain refinements such as the "double shift" and the "metaplastic shift" of the nuclear index are not encompassed by these simplified procedures

Embryologic, Morphologic, and Physiologic Objections to the Aineeth Hypothesis and Its Modifications—Aineeth's correlation of neutrophile age-increase with increase of nuclear lobulations has been criticized by many observers The primary difficulty of meaning of "age concepts" has been mentioned The varying estimates of "duration of life" of the circulating neutrophile have been referred to The lack of correlation between cell age and nuclear segmentation is emphasized by Naegeli, Bunting and others Schilling's concept of the degenerative "stab" nuclei must be classed as evidence against Aineeth's correlation hypothesis In the field of comparative (animal) leucocyte morphology there is some evidence that nuclear lobulation may vary in different species Personal observation of the blood of the young white rat indicates that the great majority of neutrophiles are either nonfilament or single filament forms (i e Class I and II of Cooke) although the nucleus may be quite tortuous The neutrophile of the monkey, *macacus rhesus* is reported by Kumbhaari¹⁵ and Hall¹⁶ as characteristically hypersegmented Hall's drawings show typical neutrophiles with nuclei composed of 5 to 6 lobes connected by very fine chromatin filaments Kumbhaari and Musser state that neutrophiles containing nuclei "with 10, 12, and 15 lobes were frequently encountered" These observations suggest that "age" is certainly not the only factor concerned in nuclear segmentation

The several modifications and "simplifications" of Aineeth's complex procedure are free from many of its fundamental objections The "modification-ists" (except Cooke) either frankly disagree with Aineeth concerning his age correlations in groups beyond Class I, or dodge the issue entirely Cooke, however, and later Cooke and Ponder go the whole way with Aineeth in linking up nuclear segmentation with cell maturity All are agreed in the general proposition that the nuclear "shift to the left" in the neutrophile formula reflects an increase in relatively young forms and constitutes an important method of study

Cooke and Ponder have presented certain experimental evidence, which if extended and confirmed, would strongly support Aineeth's major premise The reader is referred to the monograph⁵ of these authors for details It is interesting to note that Cooke and Ponder estimate "the whole length of life of a polymorph in the blood stream" at "about three weeks" (p 24 of their monograph) This contrasts sharply with the statement of Bunting that "the life of the neutrophile within the blood is measured by days and even almost by hours" Both of these statements are somewhat at variance with the usual present day assumption of a "four-day life span."

It is of further interest to note that, contrary to Cooke and Ponder the findings of Isaacs¹⁹ suggest "that when immature cells enter the blood stream they are eliminated as such and do not mature in the peripheral circulation"

From the physiologic standpoint the Aineeth hypothesis presents certain difficulties The efficiency of young neutrophiles as compared to mature neutro-

philes in defense against infection is a highly controversial subject which is beyond the scope of this presentation (see Cooke and Ponder, p 46 49) A corollary of this difficulty concerns the question of prognosis

Technical and Utilitarian Objections—Many of the technical (morphologic) difficulties of the original Aineh procedure are minimized by the application of Cooke's criterion of the filament There are other difficulties, however, which remain inherent in the method regardless of "simplifications" For example we have seen an experienced hematologic technician mistake a large number of monocytes (of the "transitional type") for supposed young (band-form) neutrophils We have not infrequently seen mononuclear cells that might be classified as metamyelocytes by one observer and "Rieder cells" by another and monocytes by another Occasionally it is difficult to decide whether a given nucleus is of filament or nonfilament type

As to the best technic of stain there is sharp divergence of opinion Schulz, Cooke and Ponder, and others recommend smears of blood on glass slides, and the employment of Giemsa stain These authors condemn the use of Wright's, Leshman's and Romanowsky's stains as "not satisfactory for the purposes of the Aineh count" Piney²⁰ recommends cover-slip preparations with modified Giemsa stain Farley and his coworkers prefer Wright's stain and cover-slip preparations In this country it seems fair to state that the fate of the neutrophile count in clinical hematology will be decided on the basis of Wright's stain (or a similar Romanowsky modification)

The utility of these procedures in clinical practice depends upon (1) the relative simplicity of execution, (2) the accuracy of results and (3) the helpfulness in diagnosis, prognosis, and treatment

The difficulties of execution of the simplified procedures are not great, and fairly accurate results may usually be obtained in the hands of experienced workers Our own observations, however, would indicate that the "mean error" of the method is considerably greater, and the "personal equation factor" much more important than is admitted by some enthusiasts (e g see Cooke and Ponder, p 8, 14) This is particularly true in many cases of serious infection in which toxic and degenerative changes in the leucocytes are profound

The diagnostic utility of these methods is very limited Such study may occasionally disclose the presence of an unsuspected infection or other obscure disturbance of organic health It is of practically no value in the differential diagnosis of infections It sometimes is helpful in calling attention to the development of untoward complications in the course of infections

The prognostic implications of the "nuclear shift" in infections are of considerable interest All observers agree that no deductions as to prognosis can be made except on the basis of repeated observations The fundamental problem is this how much of a "shift to the left" and what kind of shifts are to be viewed as "satisfactory" or "normally favorable" evidence of response to infection? There is, in our opinion, no easy answer or ready formula or infallible index The unknown individual "constitutional" peculiarities²¹ which make one person exhibit high fever or marked "leucocytosis with shift to the left" from some apparently trivial infection may in another person behave quite differently under apparently similar circumstances In our experience²² the

state of pregnancy and puerperium is one in which infections are particularly prone to produce exaggerated leucocyte responses. The same seems to be true of certain infections in infancy. From another standpoint, some infections seem to cause intense stimulation of one kind of leucocyte, other infections another kind of leucocyte, and still others cause little or no leucocyte stimulation.

Given a knowledge of the type of infection present, the age and general status of the patient, and the usual or "normal" leucocyte response to the infection, it is then quite possible to obtain, from accurate serial observations of the changes in the "nuclear index," the "stafi count" or the "nonfilament neutrophile count" some information of prognostic value.

SUMMARY AND CONCLUSIONS

1 The absolute age of the different leucocytes in health, their normal "life span" in the blood stream, and the duration of the several "stages" in their normal life cycles, are all uncertain.

2 The absolute age of leucocytes in relation to infection is also uncertain.

3 The relative age of neutrophile leucocytes in the blood stream is partly measurable, in terms of nuclear segmentation, to this limited extent: nonsegment (nonfilament) nuclears are for the most part "younger" than segmented (filament) nuclears.

4 In states of infection the proportion of relatively young forms of neutrophiles is *usually* (but not always) increased above that level which is more or less characteristic of health.

5 This increase of nonfilament forms in the differential count may *occasionally* be of some diagnostic importance in that it *may* direct attention to the existence of occult infection or to the development of an unsuspected "complication." Infection however is not the only cause of a "shift to the left."

6 The magnitude of the increase (i.e. the degree of "shift to the left") and the character of the "young forms" involved in the increase (i.e. the type of "shift") may be of some prognostic value when repeated observations are made.

7 The prognostic value of such observations is far from absolute and the "information" thus obtained is to be considered in proper relation to all the other available evidence.

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A STUDY OF THE WHITE AND DIFFERENTIAL COUNTS IN SIX UNSELECTED CASES OF INOCULATION MALARIA*

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SURPRISINGLY little has been reported on the leucocyte and differential counts in malaria, most of the work being confined to investigation of the red blood cells. It was thought that a study of these cells might prove of value in inoculation malaria, as used in the treatment of paresis, both from a clinical standpoint, and as a means of determining whether or not this inoculation malaria differs from clinical tertian malaria. It was also decided to study the so called nonfilament count with the hope of gaining more information, particularly in regard to the severity of the fever.

Craig¹ reports "As regards the white corpuscles, in general the reduction in their number corresponds with that of the red cells. During the paroxysm there is often a leucocytosis, while between the paroxysms the leucocytes are markedly reduced in number. This is in general true of all forms of malarial fever but in some cases of fatal pernicious malaria leucocytosis is observed. Much attention has been paid to a relative increase in the mononuclear leucocytes as of diagnostic importance in malarial infection. While probably in a majority of instances there is a considerable increase in this type of cell, it has not been the writer's experience that very much weight can be given in diagnosis to a mononuclear increase as it occurs in so many conditions not malarial in nature." Alteration of the leucocytes is said by one observer to be the first thing observed following inoculation. This alteration preceded the fever and even the appearance of the parasite in the blood or enlargement of the spleen. Leucocytosis is reported by Stitt² to occur only in malignant forms or in the presence

*From the Cleveland Clinic.

of complications Emerson⁹ and Thompson⁹ report a leucocytosis at the time of 11901, with a moderate leucopenia during the apyrexial period Thompson⁹ reports in addition, a leucopenia when the parasites are numerous a rise when they are few, and a marked leucocytosis when they cannot be detected Morrison⁷ notes that the leucocytes are below normal during the febrile periods and above normal after these periods

As regards the differential count, the most constant and persistent finding in malaria has been an increase in the monocytes This monocytosis is said to be pronounced in the apyrexial periods^{8,9} and least marked during the febrile periods, and is the inverse of the temperature curve^{10,11} When monocytosis is late in appearing it is preceded by a high lymphocytosis² Morris³ reports a monocytosis during the apyrexial periods as high as 90 per cent, persisting some months after the fever has subsided This monocytosis is said by many to be of value in diagnosis Bohn¹¹ believes that the blood picture is so characteristic as to be diagnostic even in the absence of the parasites Swan¹² does not believe that a large mononuclear increase is constant either in the acute stage or in carriers, but states that it, in suspected cases there is an increase it is reasonable to assume that the patient has malaria or has had it Craig,¹ on the other hand, does not believe that much importance can be attached to this finding in diagnosis

Emerson⁹ notes a relative decrease in the polymorphonuclear cells Ross finds that the polymorphonuclears, although few in number during the apyrexial periods, do not vary much from day to day Seven days after the fever there is a marked increase, and much variation

The lymphocytes apparently do not change much, although Talbot¹³ reports a relative and constant increase in the small lymphocytes, as high as 54 per cent

Hughes and Shrivaston,¹⁴ working with quinine observed that a single dose of this drug, administered intravenously, produced a leucocytosis, which they thought was due to contraction of the spleen This leucocytosis was followed by a leucopenia, the reduction in lymphocytes being most marked, although the polymorphonuclears were also reduced Oral administration caused a small increase in the leucocytes, affecting chiefly the large lymphocytes and monocytes Siperstein and Litman¹⁵ noted a rise in the white blood cell count affecting the monocytes, and showed that when quinine was combined with alkalies there was a sharp rise in both the red and white counts, which reached a maximum on the second day

Briefly then, the differential count in clinical malaria is characterized by a moderate leucopenia, with a relative decrease in polymorphonuclears, and a relative increase in monocytes, pronounced in the afebrile periods, and decreasing in the pyretic periods

Perhaps the most interesting feature of the blood picture is to be found in the so called "filament nonfilament count," or the modified Wineth polymorphonuclear count The value of the Wineth count has been known since its inception in 1904 It is common knowledge that infection causes a "shift to the left" of the polymorphonuclears, that is, an increase in young forms In health the number of nonfilament cells, or Wineth Class 1, ranges from 5 to 15 per cent

of the differential count. This count, done after the modified method of Farley, St. Clair, and Reisinger¹⁷ is simple and may be quickly calculated. It is remarkably stable in health, very sensitive to infection of all types, and according to Cooke and Ponder¹⁸ may give evidence of toxemia before the general blood picture gives any indication, and hours or even a day before the onset of clinical signs.¹⁹ Reznikoff,¹⁹ Minor and Ringer¹⁶ note the finding of an unexpectedly bad picture which was apparently not justified by the nature of the case but the reliability of which was substantiated by subsequent developments.

Cases in which the percentage of immature cells reaches 50 per cent before death are unusual. Large¹ believes a nonfilament count of 50 per cent or over carries a fatal prognosis in infections other than malaria. Koppe² made use of this count in malaria. Blood counts were taken daily at the same hour. He noted a marked shift to the left, with the Aineeth Class 1 (nonfilament) cells as high as 52 per cent and concluded that a shift to the left in the neutrophilic blood picture was the most constant finding in malaria that it persisted for a considerable time after the fever had subsided, and was of value in diagnosis. Also that an increasing shift to the left indicated a coming relapse. Macfie²³ reports a fatal case of malaria with a nonfilament count of 78 per cent. Henson²⁴ substantiated the finding of a shift to the left in infection, but stated that in malaria there was no change from the normal Aineeth picture.

Nine cases of paresis treated with malaria were investigated along these lines of which six only are reported, because these were more fully investigated. No attempt was made at selection, the cases being taken as they were admitted for treatment. All cases were inoculated by the intravenous route with benign tertian malaria from other patients. Of the six cases, two were complicated, necessitating a termination of the malaria, which was done by oral administration of quinine. In one case the fever subsided spontaneously, and in one case the parasite was never found in the blood, although the patient had two rigors similar to those of malaria, occurring too long after inoculation to be due to protein shock.

In order to keep any personal error constant, the blood for all counts was taken by me, and all counts made by me personally. From time to time these counts were checked by other workers, and in cases of disagreement, were re-counted until they checked. Two white cell counting pipettes only were used for the entire series of counts, and all total counts were made with the same dilution, using the same counting chamber for each count. The differential counts were made on carefully prepared cover slips, stained with Wright's stain, and mounted. Two hundred cells were counted in each differential count. It has been shown by Cooke and Ponder¹⁸ that the error in the nonfilament count is so small that it is sufficient to count 50 cells. In all cases reported 100 cells were counted. These are reported, not as a percentage of the differential count but as a percentage of 100 neutrophils, thus making the nonfilament count correspondingly higher than that of other observers. Twenty per cent was taken as a high normal.

When possible, counts were made before inoculation to establish a normal for the individual. All counts were made at the same hour daily, in all cases. No attempt was made to follow the chills. It will thus be seen that some counts

were made during periods of fever and some in the afebrile period. This may account for the daily variation found in all cases. The temperature of the patient at the time the count was made was noted. It was found that this bore no constant relation to the counts in any way, and hence it is not reported. The patients were followed daily as long as they remained in the hospital.

LEUCOCYTE COUNT

In all six cases there was a leucopenia, with a gradual return toward normal at the end of the infection usually beginning soon after the termination of the fever. The leucopenia was marked, two cases falling below 2000 cells. The return to normal was gradual in all cases. The leucopenia was delayed as long as forty-eight hours after inoculation. One case showed a transient rise after inoculation, followed by a fall. Both complicated cases showed a pronounced leucocytosis at the onset of the complications.

DIFFERENTIAL COUNT

A Neutrophiles—Four cases showed a rise in the neutrophile count twenty-four hours after inoculation. The count varied from day to day but all cases showed a progressive fall, except the complicated cases. Of these one showed a marked rise (to 88 per cent) the count remaining high with a slow return toward normal at the end of the stay in the hospital. In both complicated cases the neutrophile count remained higher than in uncomplicated cases. There was a marked fall at the termination of the fever in three cases. One case subsided spontaneously (without quinine) and showed the same marked drop after the abatement of fever. The fall occurred two or three days after the pyrexial period, and was abrupt, continuing low, then gradually rising toward a normal level.

B Lymphocytes—Small and large lymphocytes have been grouped together in all cases.

The lymphocytes showed the same diurnal variations. They rose gradually throughout the infection. Of the two complicated cases one continued at about the same level, and one showed a marked drop. Three cases showed a marked rise at the termination of the fever occurring on the second or third day. A fourth subsided spontaneously (without quinine) and showed the same relative lymphocytosis.

The striking feature is the approach of the neutrophile and lymphocyte curves after the fever has been terminated. This occurred in all cases except those which were complicated. It occurred in the case which subsided spontaneously, and also in the case in which no parasites were found.

C Monocytes—There was a small increase in monocytes following inoculation, which usually occurred before the appearance of the parasite in the blood, or of rigor, and was of some value in prognosticating the appearance of chills. The maximum monocytosis found in the six cases was 12 per cent. One case showed a drop about halfway through the course, the count remaining low throughout the duration of the patient's stay. One complicated case showed a material drop at the onset of complications. The lowest count was found in the case in which inoculation was unsuccessful. Three of the cases showed a con-

siderable rise after termination of the fever by quinine. One case subsided spontaneously without quinine, and showed the same rise. Two cases were not materially affected.

D Other Types—The other types of white cells showed no changes worthy of note.

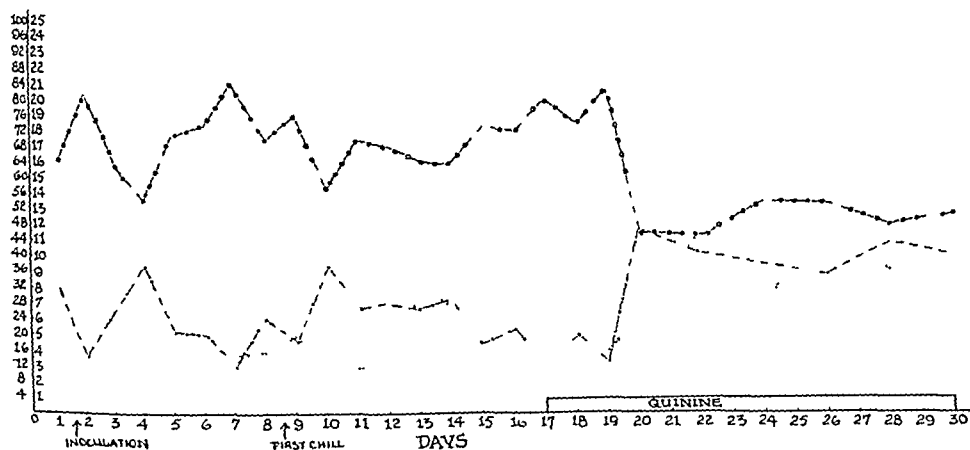


Chart 1—Differential counts in an uneventful case. The approach of the neutrophile and lymphocyte curves is clearly shown (Case 1A ——— polymorphonuclears ——— lymphocytes)

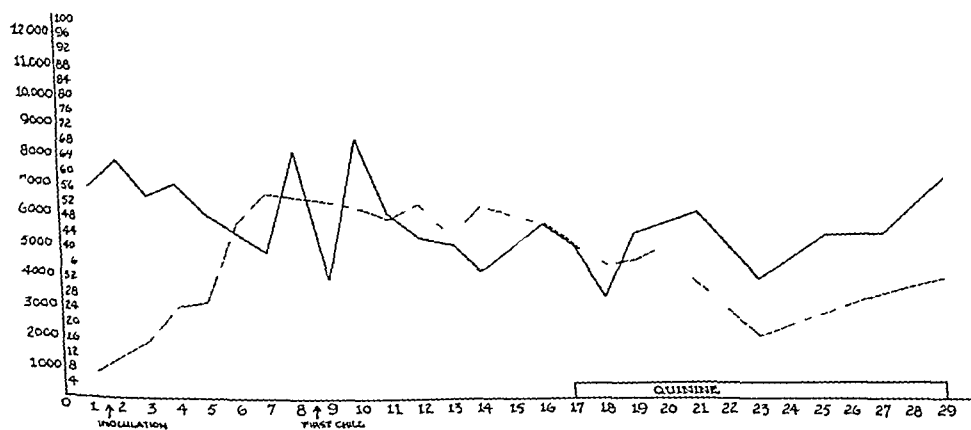


Chart 2—A graphic representation of the total leucocyte count and the nonfilament (Arneth Class 1) cells. In this case the leucopenia was not marked (Case 1B ——— white blood cells ——— nonfilament count. % 100 neutrophils)

NONFILAMENT COUNT

All cases showed a rise in nonfilament cells after inoculation. This count was remarkably constant throughout. The curve was proportional to the severity of the infection. In one complicated case the count rose as high as 88 per cent before the condition of the patient appeared serious, and before the differential count showed any material change. This is the highest count ever observed at this Clinic. In all cases the count remained high during the course of the fever with a gradual return toward normal at the termination

The drop was most marked at the termination of the febrile period. Two cases did not show this marked drop (one subsided without quinine), and the patients were discharged in good physical condition with nonfilament counts of 66 per cent and 44 per cent respectively.

In all cases where inoculation was successful as evidenced by chills and

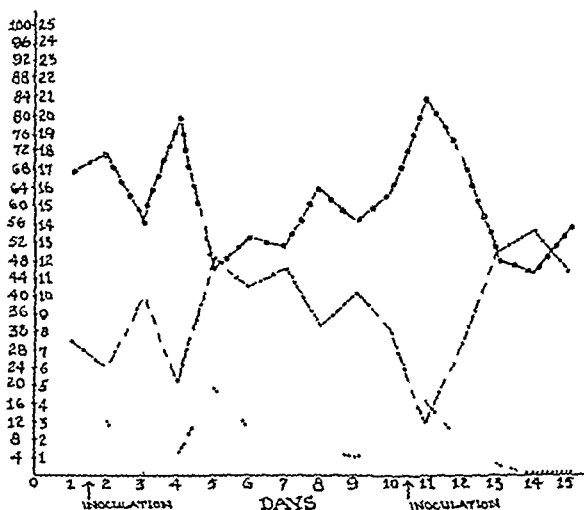


Chart 3—Inoculation was not successful in this case. The differential count does not show the same variation. The monocytes are relatively few in number. (Case II A. monocytes)

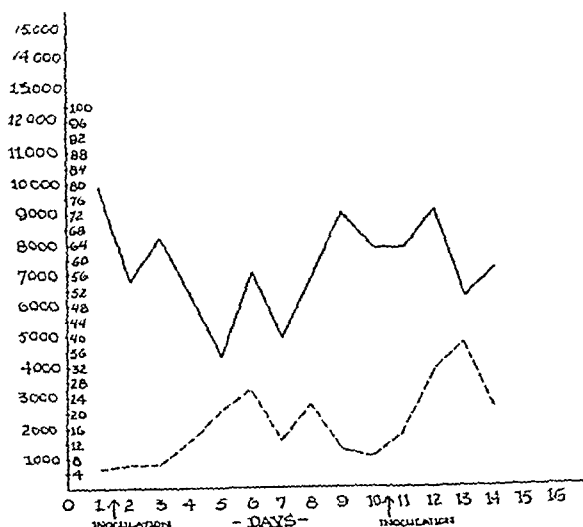


Chart 4—Leucopenia is slight, and the nonfilament count did not rise above 38 per cent. (Case IIB. ——— white blood cells ——— nonfilament count. % 100 neutrophils)

the appearance of the parasite in the blood the nonfilament count reached 50 per cent and over. The cases in which inoculation was not successful showed a maximum nonfilament count of 38 per cent.

Charts are shown of three cases. In Case 1 there was an uneventful course of fever. In Case 2 inoculation was unsuccessful after two attempts. In Case

3 the patient became so severely ill that the course of the fever had to be terminated after three chills. This was foretold by the blood picture two days before the patient's condition appeared serious.

The monocytes are plotted on a scale four times that of the other cells to make them more apparent.

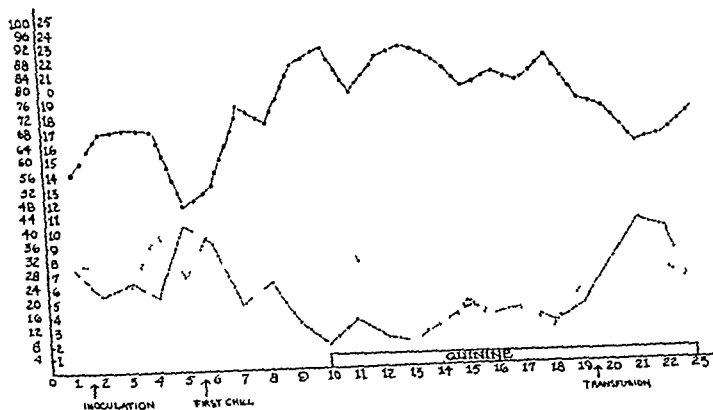


Chart 5—This patient became so ill as to necessitate termination of his course. Note the increase in neutrophils with a corresponding drop in lymphocytes, which was not apparent until the seventh day. Note also that these two curves never meet (Case IIIA). — 0 — 0 — 0 — 0 — polymorphonuclears — — — — — lymphocytes
monocytes)

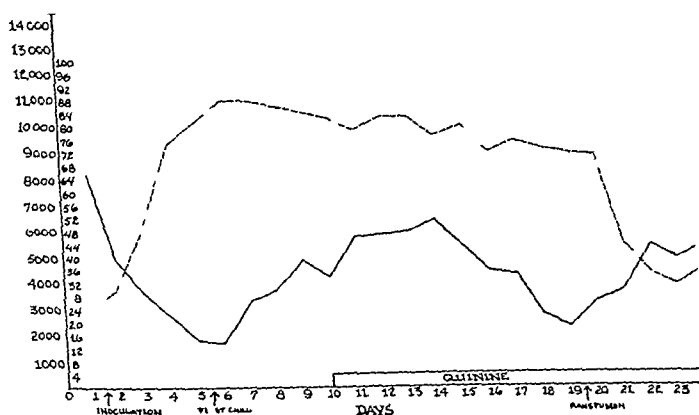


Chart 6—The leucopenia is more marked. The nonfilament count rises abruptly to reach a maximum of 88 per cent on the sixth day twenty-four hours before an appreciable change is noted in the differential count. Transfusion on the nineteenth day cut the nonfilament count in half and more than doubled the white blood cells. Recovery was uneventful (Case IIIB). — — — — — white blood cells — — — — — nonfilament count % 100 neutrophils)

SUMMARY

- 1 Daily white differential and nonfilament counts are reported in six unselected cases of inoculation malaria.
- 2 The blood picture in inoculation malaria differs materially from that in clinical tertian malaria.
- 3 Leucopenia is present throughout the course in uncomplicated cases, often marked, with a gradual rise toward normal after the infection has terminated.

- 4 The neutrophiles fall gradually, and show a marked fall at the termination of the infection
- 5 The lymphocytes show a slight rise throughout the course with a sharp rise at the termination
- 6 The monocytes show a moderate increase which usually occurs before the appearance of the parasite in the blood
- 7 Other types of cells are not materially affected
- 8 There is a marked increase in the nonfilament count, which is proportional to the severity of the infection. The count remains high throughout. It is of value in prognosis and diagnosis
- 9 There is a noticeable difference in the blood picture in complicated cases
- 10 Inasmuch as these changes occurred in all cases it is doubtful whether quinine has played any part in their appearance

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A REVIEW OF GRANULOCYTOPENIA (AGRANULOCYTOSIS)*

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THE term granulocytopenia more correctly expresses the actual condition existing in that disease known more commonly as agranulocytosis than any that has been suggested. It has the further virtue of being consistent with the accepted nomenclature describing variations in numbers and percentages of white blood cells. Any term that is expressive of the essential pathology in agranulocytosis should be adopted and furthermore, it should possess the added virtues of simplicity and pronounceability. The term agranulocytosis in its strict sense, means "an increased number of immature granular cells," whereas granulopenia expresses "a decrease of the granular cells." The term granulopenia fits the classification proposed by Boerner,¹ which should be adopted and which is given below:

Neutropenia—A decrease in neutrophils
Basopenia—A decrease in basophils
Eosinopenia—A decrease in eosinophils
Lymphopenia—A decrease in lymphocytes
Monopenia—A decrease in monocytes
Granulopenia—A decrease in granular cells
Thrombopenia—A decrease in thrombocytes

I would suggest the addition of the last two terms in the above table as fitting the classification and expressive of the pathology in agranulocytosis.

Granulopenia seems to be a disease which may be acute or chronic, severe or mild depending upon the extent to which the granular cells are decreased. The condition known as agranulocytosis, is probably the most extreme state of the disease in which the granular cells practically completely disappear from the peripheral blood, this, in turn, being followed by local or general sepsis, and usually by death.

A mild or chronic granulopenia undoubtedly exists in some patients, and may be responsible for the existence of certain symptoms and syndromes. Roberts and I have attempted to describe a variable clinical condition which has its cause in a decreased number of granulocytes. This condition seems to be characterized by weakness, easy tiring, tendency to fatigue and chronic exhaustion, the severity of these symptoms being dependent upon the extent of decrease in the granulocytes. We were able to show from a study of 8000 records from private practice that the typical granulopenic patients are usually women between the ages of forty and sixty. It is also this type of patient in which agranulocytosis is most often seen, and one who has a chronic granulopenia should always be regarded as a potential candidate for the development of the acute attack.

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Acute granulopenia, in which the white cell count may fall as low as 2000 with 25 per cent neutrophils, is characterized by a somewhat sudden onset of lassitude, weakness, a tendency to sleep, and frequently the development of ulcers in the oral cavity, or in any area of the body that is normally inhabited by bacteria. These bacteria may be nonpathogenic under normal conditions, and so long as the individual is protected by the daily conferred immunity of the granulocytes, but when this is decreased, they invade the tissues, producing ulcers of various types. It is probable that the development of ulcers and other evidences of infection stimulates the bone marrow to renewed activity and thus aids in the recovery of the patient.

I will now discuss in detail the subject of agranulocytosis which might more correctly be termed acute fulminant granulopenia.

HISTORICAL

The disease now known as agranulocytosis was first described in detail and given a name by Schultz² in 1922. There is little doubt, however, that it had existed for many years prior to that date since Senator⁴ described four patients with fatal pharyngitis in 1888, and Brown,⁵ in 1902, described a case of "acute primary pharyngitis with extreme leucopenia," in which the white cell count was 200. Furthermore, Baldridge and Needles⁶ have found a case of undoubted agranulocytosis in the records of the University of Iowa Hospital in 1910. Larson and Barion⁷ in 1913 reported "a case in which the fusiform bacillus was isolated from the blood stream." Their patient died after running a septic course with ulceration of the upper jaw, with a white cell count of 2400 and fusiform bacilli in the blood stream. I feel that they were dealing with what we now regard as agranulocytosis, and their report is of considerable importance due to the association of the fusiform bacillus, an organism that must be regarded with suspicion as an etiologic agent in this disease.

An eighteen-year-old boy with recurrent agranulocytosis has recently been described by Rutledge, et al.⁸ and they refer to Leale's⁹ report of the same patient when he was an infant. It is probable that the disease has existed for many years before Schultz's first report and it is also probably true that the incidence has increased considerably since 1922. Whether this increase is due to an actually increased number of cases or to the fact that the attention of the medical world has been called to the condition cannot be determined, though the former view is probably correct.

Various names have been applied to the condition as, "Angina agranulocytica" by Friedmann,¹⁰ "mucositis necroticans agranulocytica" by Weiss,¹¹ "sepsis with granulocytopenia" by David,¹² "malignant neutropenia" by Schilling,¹³ and a recent editorial in *The Journal of the American Medical Association*¹⁴ refers to the condition as "granulocytopenia." The term, "agranulocytosis," is still in current use, but the term, "agranulocytic angina," should be abandoned, since many of these patients show no oral lesions. However, it may be applied to that class of patients showing oral lesions, as they usually die of the overwhelming infection, whereas the acute, fulminating type seem to die from the mere absence of neutrophils without showing any evidence of infectious processes.

CLASSIFICATION OF GRANULOCYTOPLNIC CONDITIONS

1 Agranulotoxicosis, in which the neutrophils may entirely disappear, usually following chemical poisons, as benzene and arsenic Weiskotten¹⁵ has demonstrated the toxic effect of benzene on the bone marrow as regards its capability of producing neutrophils, while Talley and Griffith¹⁶ reported a case in a negro following arsphenamine therapy Mme Pouzin-Maleguez¹⁷ reports a similar instance and ascribes arsenical therapy in syphilitics as being responsible for at least eight collected cases The depressing action of arsenic on the granular elements of the bone marrow has been shown by Dodd and Wilkinson,¹⁸ Wheelahan¹⁹ and Farley²⁰ McCord²¹ brings out the depressive action of benzene in an excellent paper I²² have been able to produce experimental agranulocytosis in rabbits by the subcutaneous and intraperitoneal injection of small doses of benzene over long periods, in which the animals developed first a chronic granulopemia, this being followed by an acute attack with mouth ulcers, sepsis, and death The red blood cells and platelets were unaffected, and the conclusion was evident "that the smaller the dose of benzene, the more selective is its affinity for the myeloblastic tissues" It is very likely that this class of substances is responsible for some of these cases

2 Agranuloradiation, in which the bone marrow is temporarily depressed following excessive dosage of x-ray therapy This happened in a patient I recently reported²³ and also involved the red cells as well as the granular cells Clark²⁴ states that radiologists are always aware of this effect due to excessive x-ray dosage, for which reason extreme caution should be used in the regulation of the dosage in the treatment of patients with low white cell counts Waters²⁵ makes the pertinent comment regarding the effect of x-ray on the tissues "small doses stimulate, large doses destroy"

3 Agranulosepsis, a neutropenia due to the effect of unusual bacterial toxins or to an unusual response of the bone marrow to ordinary bacteria Of the many bacteria isolated from the oral lesions and the blood stream of these patients, the *Bacillus pyocyaneus* has been found in a number of cases, and it is claimed that the introduction of this organism into laboratory animals will provoke a marked leucopenia (Lovett,²⁶ Lanthierum,²⁷ Windham,²⁸ Keeney²⁹) Roberts and I,³⁰ however, were unable to produce a leucopenia with a *Streptococcus hemolyticus* isolated from the blood stream of a typical, fulminating case Norris³¹ states that his recently isolated *saccharomycete* produces a marked leucopenia in laboratory animals

It is probable that many of these patients should fall into this class, it may be possible that the large number who give a history of oral infection prior to their attack of neutropenia had a depression of the bone marrow due to invasion by mouth organisms Such cases have been reported by Cannon,³² Dyer and Helwig,³³ Whitehead,³⁴ Call, Gray, and Hodges,³⁵ Kastlin,³⁶ Thomson,³⁷ Moore and Weider,³⁸ Skiles,³⁹ Talley and Griffith,⁴⁰ Blumberg and Peace,⁴¹ Kracke,²³ and others Bacterial toxins or dead bacteria could possibly produce a neutropenia, as suggested by Bromberg and Murphy's⁴² patient developing a marked neutrophilic leucopenia following typhoid prophylaxis

4 Agranulocytosis, apparently a disease entity in which an unknown agent produces a depression of the bone marrow, this resulting in loss of neutrophilic

resistance, with subsequent overwhelming infection in those areas of the body normally inhabited by bacteria. This class should also include that type of case which is characterized by loss of neutrophils and death without evidence of infection.

5. Aleucemic lymphatic leucemia, in which the granulocytes may almost disappear from the peripheral blood.

6. Acute infectious diseases associated with leucopenia which is often neutrophilic in type, or a relative lymphocytosis with a low total white cell count. This blood picture may be due to an actual failure of the bone marrow to produce the normal number of neutrophils. These diseases include typhoid, typhus, measles, mumps, malaria, influenza, dengue, and sometimes syphilis and tuberculosis.

7. Roseola infantum, a neutropenia of infancy, presenting a definite clinical syndrome, but which is not necessarily fatal.

ETIOLOGY

The etiology of agranulocytosis is unknown though much work has been done bearing on the sequence of events in the clinical course. The question was first brought up by Schultz³ as to whether the disease is primarily an unknown infection, resulting in bone marrow depression (this in turn followed by overwhelming infection) or whether an unknown chemical agent is responsible for the bone marrow failure to produce granulocytes.

In a consideration of the possible etiologic factors Kastlin⁴³ asks the pertinent questions. Is the disappearance of the neutrophils due to

1. an increased peripheral destruction?
2. an abnormal distribution of the cells?
3. failure of cell development?

Up to this time there is little or no evidence to indicate that increased peripheral destruction of the granulocytes is taking place. The spleen is seldom enlarged, and the red cells are little disturbed. Roberts and I⁴⁰ were unable to demonstrate a circulating agent which showed evidence of toxicity for the neutrophils. In this test we mixed 2 cc. of the patient's blood with an equal quantity of normal blood of the same type. Two cubic centimeters of the latter was then mixed with an equal quantity of a second normal blood, this serving as a control. Both were incubated, and total and differential white cell counts were made at two hour intervals for forty-eight hours. No differences were observed, though survival studies (which might have given additional information) were not carried out.

The blood of patients with agranulocytosis has been injected into laboratory animals, but no disturbance of the blood picture resulted.

There is little evidence to assume that the absence of the granulocytes is due to an abnormal distribution of the cells. I have checked both venous and capillary blood on the ten cases that I have seen and the counts were essentially the same. There is little doubt but that the abnormal distribution of cells often affects the total white cell count, due to the so called "shifting of the vascular

bed," as is being so well demonstrated by Garvey.⁴¹ However, in these instances the relative percentage of neutrophils is little affected.

It has been well demonstrated that the failure to observe granulocytes in these patients is due to a failure of cell development, since the bone marrow at autopsy has been found consistently poor in granular cell elements, with erythroblasts present to a normal degree. Piette⁴² has described these findings in detail. Moreover, studies of the bone marrow have been made during the life of the patient by Buck,⁴³ Zodek,⁴⁴ and Schultz and Jacobwitz.⁴⁵ They all agree that the bone marrow function is depressed.

From a summary of evidence to date, I believe it can be reasonably concluded that agranulocytosis is primarily a dysfunction of the bone marrow and, as suggested by Roberts and myself,³⁰ this is then followed about four days later by the disappearance of the granulocytes in the peripheral blood. Since the normal life span of the neutrophil is about four days,¹² we were able to show that the blood stream changes were manifest about seventy-two hours prior to the clinical onset. Similar observations have been made by Ehmann and Pieuss,⁴⁶ Bantz,⁴⁰ Hunter,⁴⁷ and Lauter,⁴⁸ Ashworth and Maphis,⁴⁹ and Kracke.²³ Rutledge, Hansen-Pruss, and Thayer,⁸ in carefully studying a case of recurrent agranulocytosis, noted the decreased neutrophils prior to the clinical onset during a large number of attacks.

Assuming that agranulocytosis is primarily a dysfunction of the bone marrow, the next point in consideration of the etiology is the nature of the unknown substance responsible for this depression.

As indicated before some of the substances brought out under the heading of agranulotoxicosis may be responsible, these including arsenicals, bacterial toxins, dead bacteria, benzene and its allied and related products. This has been well discussed by Jacobsen.⁴¹ The fact that about 75 per cent of these cases occur in women between the fourth and sixth decades, that the disease was first reported as a clinical entity in 1922, that about 90 per cent are reported from Germany and the United States, and that benzene products probably have their widest usage in those two countries, lends further support to the possible etiologic factor lying in that field.

All who have the opportunity to study these cases should carry on thorough bacteriologic and hematologic laboratory studies so as to add further information bearing on the etiology and, in particular, should obtain thorough and complete histories with especial reference to the use of unusual foods, drugs, cosmetics, clothing, shoe and hair dyes, and other substances of a rare nature that may contain bone marrow depressing substances, as benzene or its allied products.

The large number of cases giving a history of oral sepsis or tooth extractions prior to the onset should be carefully investigated as to drugs, chemicals, mouth washes or local treatment used during that period. Shear⁵⁰ believes that oral sepsis, and Vincent's organisms in particular, is the cause of the disease. He cites the frequent occurrence of mouth lesions in the disease and the increased incidence of Vincent's angina since the war. At Emory University we are now carrying on extensive blood work on a large series of that class of patients.

INCIDENCE

The disease occurs chiefly in middle-aged women, usually in the fourth or fifth decade with a ratio of four women to one man. Hodges⁶ reports three cases in males, while Hutcheson⁷ reports two males in five cases. There is no age limitation, as evidenced by Jacobsen's⁴ report of an infant, Bantz's⁵⁰ case in a boy of four, Dyer and Helwig's³³ case in a boy of six. Gordon⁵³ describes a woman of sixty-six, and Roberts and I³⁰ a woman of seventy-two years.

The disease is apparently not contagious or infectious, though Hait⁹ describes three cases in one family. So far as I know, there is no other recorded similar instance. It has no seasonal variation but does have geographic limitations. About 80 per cent of the cases have been reported from Germany and the United States. At this time I have found only two cases from England, reported by Gairrod⁶⁰ and Batten.⁶¹ Reports from Italy are quite numerous and in 1929 Yarin⁶² stated that only three cases had appeared in the Russian literature. A small boy has died with the disease in Japan and Bakker and Kuiper⁶³ report one from the Dutch Indies.

It seems to occur in all classes of life and occupation, having been reported in school children, housewives, business and professional women, laborers, scrub women, physicians, farmers, prostitutes, and ladies and gentlemen of leisure, it seems peculiarly prevalent in the last named class. For some reason the incidence of the disease is high among physicians and nurses. The literature affords many examples of this. In the eight cases of Harkins⁶⁴ was one doctor, one medical student, one nurse. In the ten cases I have seen was one doctor and two nurses. Reports of the disease in nurses and physicians have been made by Logefiel,⁶⁵ Fisher,⁶⁶ and others. It is confined largely to the white race. Talley and Griffith,¹⁶ however, report one case in a colored female, aged thirty-six, who had been taking asphenamine. There seems to be no occupational etiologic factor.

SYMPTOMS AND CLINICAL COURSE

There seem to be four distinct clinical types as follows:

- 1 Acute, fulminant type with no infection
- 2 Acute, fulminant type with localized or generalized infection
- 3 Chronic type with acute remissions
- 4 Chronic type with no acute attacks

The acute attack sometimes begins with the patient having a prodromal period of two or three days of weakness, lassitude, malaise, and perhaps sore throat. Some have no prodromal period, the attack occurs suddenly without warning. The outstanding feature is the extreme weakness and almost complete prostration. One patient stated that she had no pain, but was so weak that "she could hardly raise her head from the pillow." They usually suffer no discomfort, unless there are ulcerations of the oral cavity. Frequently, there is a marked dysphagia and varying degrees of edema of the cervical tissues. There may be ulcers on the anus or vagina, but in some cases no ulcerations are found in any locality. Jaundice is present in about 40 per cent and Hueper⁶⁷ noted gallbladder disease in three out of seven cases. Aside from the ulcera-

tions, there are usually no positive physical findings. There is an increased pulse rate, and increased temperature may be found in those who have evidence of infection. The lungs are normal except in those who die of a terminal bronchopneumonia.

The course varies. A patient may live to show evidence of blood stream and localized infection, again, death may occur within two or three days, apparently from terminal pneumonia.

The acute, fulminant cases usually die, but, if one recovers from an attack, the course then becomes chronic (with the probability of a future remission) and the blood count slowly returns to a low level for normal with the neutrophilic percentage always low. Remissions have been observed for as long as two years,⁹ but the usual time is from one to three months. Because of the probability of remissions, the reporting of recovered cases should be done with considerable reservation.

As brought out before, there also exists a chronic type of neutropenia which may never show evidence of an acute attack. The patient may have a white cell count as low as 1000, with the neutrophiles almost or entirely disappeared from the peripheral blood, and the average white cell count may range around 2000 to 3000. Such a patient, who is a physician⁶⁸ has told me that on days when his white cell count is as low as 2000, he is so weak, tired, and depressed that he is unable to go to his office. On one occasion his count fell to 800 with complete absence of neutrophiles, and he had a typical attack of agranulocytosis. It is also probably true that many individuals have mild attacks of weakness, loss of activity, and depression because of a lowered number of leucocytes. I have recently had under observation a colleague who is in splendid health, but for a period of four or five days felt weak, sleepy, tired and depressed. During this period the leucocyte count averaged 4500 with 40 per cent neutrophiles, consisting of juvenile, band, and young segmented types. At the same time there was noted a mouth ulcer, which promptly healed when the leucocyte count reached an average of 7000 to 8000.

LABORATORY FINDINGS

The findings of greatest diagnostic value are those of the blood, in which there is a marked neutropenia with later involvement of the lymphocytes and monocytes.

The white cell count may fall as low as 100 cells per cmm with the total absence of granulocytes. However, a count of 10,000 with complete absence of granulocytes has been observed. I have seen one patient with a white cell count of 7000 and all lymphocytes and monocytes. The red cells and hemoglobin are little affected, except in patients whose illness is prolonged.

THROMBOPENIC GRANULOCYTOPENIA

There is often a hemorrhagic diathesis. In defining true agranulocytosis in which the original diagnostic criteria of Schultz are adhered to, then those cases showing a hemorrhagic diathesis must be excluded. However, more and more reports of associated hemorrhages, due in most instances to diminished or

absent platelets, are appearing. It is becoming evident that we can draw no hard and fast line between those cases showing neutropenia only and those showing the same condition complicated by diminished platelets, purpura, bleeding, etc. It is further evidence that in some individuals only the myeloblastic tissues are affected, in some the thrombocytopenic tissues are affected, and in some the erythroblastic are affected or that there may occur combinations and variations in severity of any or all of the three. In the last two cases I have studied the outstanding clinical factors were multiple, generalized and profuse hemorrhages. Yet the leucocyte counts in each case were below 1000. The platelets were almost absent. The red cells hovered around 2,000,000. Therefore, all of the elements of the bone marrow were affected, and yet it cannot be denied that agranulocytosis was a predominant factor.

I have recently studied a patient at the U. S. Naval Hospital, Washington, D. C., whose white cell count has been around 2000, platelets markedly diminished, purpura and bleeding, but the red cell count relatively unaffected. Such a patient does not present the picture of true agranulocytosis as originally described, nor could it be classified as aplastic anemia. Hence, I would suggest that those patients showing an involvement of the myeloblastic tissues and the thrombocytopenic tissues, but showing no anemia be placed in a separate classification. For this, I would propose the name thrombopenic granulocytopenia.

The classification, proposed name and differentiation from the so called idiopathic purpura becomes of real and practical importance, since in the latter condition, splenectomy is a rational therapeutic procedure, whereas in thrombopenic granulocytopenia it is obvious that splenectomy would be of little value in a condition whose pathology lies in the inability of the bone marrow to produce the normal number of platelets. In those cases with diminished platelets and purpura, with the white cell count bordering on real agranulocytosis, it can be reasonably concluded that the same toxin that is affecting the production of one is also affecting the production of the other. This differentiation of the two types of purpura may explain why splenectomy sometimes fails to be of therapeutic value in cases diagnosed as simple idiopathic purpura hemorrhagica.

BACTERIOLOGY

Many types of bacteria have been isolated from the ulcerated areas these including *B. pyocyaneus* by Dasse,²⁹ Lovett,³⁰ Keener,³¹ and others, *Streptococcus hemolyticus*,³⁰ Vincent's organisms, staphylococci and various types of gram-negative and gram-positive bacilli.

Blood cultures have been positive in about 10 per cent of the cases the organisms isolated including pneumococcus (Type 3),⁷⁰ *B. coli communis*,¹ *Streptococcus hemolyticus*,³⁰ *Streptococcus viridans*,³² *B. Acid-lacti*, *B. lactis aerogenes*,³⁰ and others. In one case I was able to isolate the *Streptococcus hemolyticus* from the blood stream, oral lesions, urine, sputum, feces, and multiple embolic abscesses.

Various organisms, including all of those named above, have been found in the heart's blood at autopsy. Plette¹⁴ found multiple bacterial emboli throughout the kidneys and stomach wall, and they have been noted in other organs as

well. As a result of decreased neutrophilic resistance, widespread bacterial invasion from the gastrointestinal tract is to be expected.

PATHOLOGY

In the acute fulminant cases there may be no demonstrable pathologic lesions except the characteristic changes in the marrow of the long bones. There is a red marrow which has been referred to as liquefied, evidently due to the absence of the supportive elements of the granulocytes. The granulocytes are decreased or entirely absent, even to the myelocytic forms. The erythroblasts and erythrocytes, however, are present to a normal degree, thereby accounting for the normal color. Such findings further support the theory of the bone marrow dysfunction rôle in producing the disease. No bacteria have been found in the marrow, except those distributed throughout the body, and none of those were capable of reproducing the disease in laboratory animals.

The necrotic lesions of the mouth, anus or cervix present essentially the same picture, that is, they are characterized by the absence of a surrounding inflammatory zone, since neutrophils are not available to form it. They may vary widely in extent, ranging from a few small superficial ulcerations to extensive gangrenous processes. Edema is often present in varying degree in the cervical tissues.

The lungs frequently show subpleural hemorrhages with areas of pleural fibrinous exudate overlying small consolidated areas which contain red cells and perhaps bacteria, but no leucocytes. The digestive tract may have ulcerations throughout its entire length.

In the acute cases, complicated by generalized infection, the heart may show the changes of toxic myocarditis, and the liver show the changes incident to septic processes, including cloudy swelling, varying degrees of fatty degeneration, and occasionally small multiple areas of necrosis. Bacterial emboli may be widespread. The spleen is enlarged, dark red, and firm, the kidneys show evidence of cloudy swelling and may present the usual changes of acute nephritis due to bacterial toxins.

Throughout the body the inflammatory changes are characterized by an outpouring of lymphocytes and plasma cells, accompanied by fluid, with the absence of the granulocytes.

The pathologic findings vary widely, being dependent upon the duration of the disease and whether or not infection has become generalized.

TREATMENT

The treatment of agranulocytosis has been generally unsuccessful. Various antiseptic agents, as alcohol, tincture of iodine, mercuriochrome, iodoform, asphenamine, silver nitrate solutions and others, have been used on the ulcerations but none of these seem to affect the course of the disease.

Various agents have been injected intravenously, as antistreptococcus serum, diphtheria antitoxin, nonspecific proteins, whole blood, autogenous and stock vaccines but none is effective. Gordon¹ claims good results with the daily subcutaneous injection of nucleic acid. Reznikoff² has used daily intravenous

injections of 0.5 gm of adenine sulphate in 25 cc of saline and claims three cures. All three, however, received other therapy, including transfusions. He further states that the same therapy was used in six leucopenic pneumonia patients, and four of these died.

The most widely used and probably the most valuable therapeutic procedure is frequent transfusions. The addition of new blood will serve not only to supply the needed granulocytes, but some believe will stimulate the bone marrow to further production. Certainly, the use of repeated transfusions is based on more scientific facts than is any other procedure.

Fisher⁶⁶ was the first to transfuse the blood of a recovered patient in the treatment of the disease. Although the white count was down to 600 with associated purpura, the patient showed evidences of recovery immediately following the transfusion, which evidences did not follow the previous use of transfusions of ordinary blood. Chrisman and Hinton⁷³ have reported three cures in as many patients by the same procedure. It is indeed fortunate if recovered patients are available for this purpose.

Many believe that the use of stimulating doses of x-ray to the long bones is of value, though caution must be observed against the use of excessive dosage, in which case actual damage is done. Friedmann⁷⁴ has been the chief exponent of x-ray therapy. He reported 43 cases treated exclusively by x-ray. He states that 23 died of complicating sepsis, that five others were moribund, thus leaving 15 with 13 actual recoveries. Analysis of his report shows that actually, about one out of three recovered. Waters⁷⁵ reports four recoveries in five patients treated with x-ray and other measures. I believe it to be of value if cautiously given in small stimulating doses by an expert with careful checking of the blood picture. Hueper⁶⁷ has proposed that gall bladder drainage be instituted in those cases that have a definite history of gall bladder disease, but so few give such a history that the use of the procedure has application to few cases, even in those, the wisdom of any surgical procedure should be questioned.

Any agent that is thought to be capable of stimulating the bone marrow to production of granular cells is worthy of a trial. Among these may be mentioned various nonspecific protein substances, vaccines, sera, adenine sulphate, nucleic acid, sera of various types, milks, etc. The intravenous injection of dead typhoid bacilli may be useful. Recently marketed preparations of colloidal sulphur have been shown to increase the leucocyte count from normal to as high as 30,000 in syphilitics. It is worthy of a trial. The production of sterile abscesses with turpentine has been done by Roberts and myself.

At this time the treatment is only palliative, there is nothing specific that can be used in view of the fact that the etiology is unknown. Keilty⁷⁶ states that he observed about 12 cases in a series of 5000 cases of oral sepsis, and that all recovered with appropriate local treatment to the mouth lesions. However, I doubt the correctness of the diagnosis in his series since he stated that no differential counts were available.

One writer states that he has used massive doses of liver extract by mouth with recovery of the patient, in spite of the fact that the white cell count was below 1000 with complete absence of the neutrophils. Since the stimulating effect of this substance on the erythroblastic function of the bone marrow is well

known, it would seem that this form of therapy is founded on rational grounds. It certainly can do little, if any, harm.

The substance sadly needed for treatment of agranulocytosis is obviously an agent that is capable of stimulating the bone marrow and that will not damage the body tissues. Such an agent has long been sought, but is not yet available. It is equally true in this disease, as in many others, that the efficiency of any treatment is inversely proportional to the number of treatments that have been used.

From a summary of present knowledge, it seems that treatment should be based on the following lines:

- 1 Frequent transfusions (immune transfusions, if possible)
- 2 Stimulating doses of x-ray to the long bones, with careful checking of the changes in the blood picture
- 3 Colloidal sulphur subcutaneously
- 4 Massive doses of liver extract by mouth
- 5 Use of local antiseptics on the ulcerations
- 6 Symptomatic treatment, with particular reference to cardiac stimulation

PROGNOSIS

Approximately 250 cases have been reported with a mortality of 85 per cent. The 10 cases that I have seen are dead. Mandelbaum⁶ recently reported 4 deaths in as many patients. Rosenthal^{77, 78} has stressed the point that, in patients with counts below 1000, death will likely occur, and in those with counts above that figure, the chance for recovery is good. This observation was based on 5 deaths and 5 recoveries in his series of 10 patients. It is probably true that the prognosis depends, to a large degree, upon the severity of the neutropenia, though Wyatt⁷⁹ reported a white cell count of 700 with recovery, and Call, Gray and Hodges,³⁰ one of 640. I⁸⁰ have recently reported a patient with a white cell count of 470, followed by temporary recovery of two and one-half months duration. However, this patient died of a third attack. Therefore it is well to bear in mind the tendency of the recovered patients toward remission. If final reports on these patients were available, I believe that the mortality rate would appreciably increase.

CONCLUSIONS

- 1 A classification of granulocytopenic conditions, based on probable etiologic factors, is suggested.
- 2 A clinical classification of the disease is suggested.
- 3 The condition is briefly reviewed, with particular reference to possible etiologic factors.
- 4 Accumulated evidence points to the disease being primarily a bone marrow depression, followed in some instances by localized or generalized infection, or both.
- 5 It is apparently a clinical entity.
- 6 The name thrombopenic granulocytopenia is proposed for those cases complicated by diminished platelets and hemorrhages.
- 7 The treatment seems to be of little value and the prognosis remains essentially as poor as when it was first reported.

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PRESENT STATUS OF THE STUDY AND TREATMENT OF LEUCEMIA*

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LEUCEMIA is, at present, a type of disease which has received much study, but which is still an unsolved problem. Although it is a comparatively rare disease, it is of importance as at its basis is the very fundamental problem of blood production, as well as the question of the nature, therapy, and abnormal physiology of neoplastic disease. The following review summarizes a few selected points, which may be suggestive for future research.

Etiology—The cause of leucemia is still unknown. There are many features which suggest a relationship to cancer, inasmuch as a type of body cell appears to lose its ability to mature.^{1 2} In this respect it is somewhat suggestive of the process which affects the red blood cells in pernicious anemia. Some of the neoplastic characters of the white blood cells in leucemia are the uncontrolled growth, the tendency to form secondary foci of growth (metastasis), the progress to a fatal termination with cachexia, the neoplastic type of metabolic rate of the cells,³ the maturation with roentgen ray irradiation, the failure to transmit the disease by inoculation of human beings with the blood of affected patients, the absence of bacteriologic data of an etiologic nature, and the birth of perfectly normal children by leucemic mothers. The cells appear to have a different chromosome number from normal body cells, which may account for their altered growth potentialities. The leucemia cells appear to be physiologically different from the type of cell to which they belong. Thus, while lymphocytes are considered as being associated with immunologic processes in tuberculosis, a fulminating form of this disease may be present with lymphatic leucemia.⁴ Leucemia-like blood pictures occur in lower animals,⁵ and a type associated with an inoculable agent occurs in fowls.^{6 7}

Symptoms—The first symptom of leucemia is ease of fatigue. The symptoms as a whole, fall into four groups: those associated with an increase in the basal metabolic rate (nervousness, abnormal perspiration, loss of weight), those associated with enlarged organs and glands (pressure symptoms, pains, cough, diarrhea, constipation, frequency of urination, and similar symptoms on the part of any organ the function of which is disturbed because of the abnormal growth), symptoms associated with anemia and myocardial insufficiency (dyspnea, edema, fatigue), symptoms associated with abnormal metabolism following the gradual progress of the disease (cachexia). The progressive anemia and, in lymphatic and acute leucemias, the blood platelet deficiency, are the result of crowding out of the formative cells by the leucemic tissue. Since the present treatment is symptomatic, it follows these four lines.

Types of Leucemia—Any of the cells of the hemopoietic system may be involved in the process—myeloid, lymphatic, monocytic, plasma cells, as well as tumor cells of various kinds. In neoplasms of some organs, very few of the cells

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enter the blood stream, whereas in others the number in the peripheral circulation is appreciable. The more advanced the stage of the disease process in the patient, the more primitive is the dominant cell in the bone marrow, lymph nodes, and blood. As the primitive cells of most of the types of leucemia are small cells with round nuclei, scanty, basophilic cytoplasm, reticular chromatin, and nucleoli, there is often some confusion in identifying their class. In acute leucemias this type of cell may predominate, and because of a superficial resemblance to small lymphocytes, they are occasionally called lymphatic leucemia, whereas the acute myeloid type is probably the more common. The change from a chronic myelogenous leucemia to the myeloblastic type at the end of the disease has often given rise to the impression that a myelogenous leucemia has turned into one of the lymphatic type. All leucemia patients, if they live long enough, end up in the "blast" stage, that is, the cells appear in less and less mature stages in the blood stream and the more immature forms are seen in greater numbers in the blood forming organs. When the blood forming cells remain confined to the bone marrow and lymphoid tissue, but few of the immature cells reach the peripheral circulation (aleucemic leucemia). When the metastatic foci, however, develop in other organs, especially those that are in constant motion, many immature forms are dislodged and the white blood cell count rises and the typical leucemic picture is seen.⁸ The disease may run an aleucemic course and terminate with a high leucocyte count, the increased numbers sometimes appearing very shortly before death. With any type of leucemia, the appearance of increasing numbers of primitive cell forms (primitive "blasts") heralds a bad prognosis.

Treatment—The therapy of leucemia, at present, is purely a treatment of symptoms. The treatment, especially of the myelogenous type, with forms of arsenic (Fowler's solution) was the method in most common use before the advent of the roentgen rays. After the general use of irradiation, arsenic fell into relative disuse, but recently attention has once more been called to its value.⁹ Benzol has had a somewhat similar history, but its effects are less easily controlled and aplasia of the red bone marrow is a theoretic, if not actual danger, at least in certain individuals.

The use of the roentgen ray or of radon has frequently been misapplied. There is occasionally a feeling that this type of radiation "kills" cells, implying a toxic or necrotizing effect. With blood cells, however, the action is of a different nature, at least with therapeutic doses. Myelocytes, metamyelocytes, young polymorphonuclear leucocytes, medium sized and small lymphocytes are stimulated to go through the rest of the stages of their normal life process, and to die of senility. This elapse of time, between the day of application of the radiation, and the time when the cells are eliminated, is often called the latent period after roentgen rays. The action on primitive myeloblasts, lymphoblasts or monocyte blasts, or the cells in the "blast" stage, is quite different. These cells are apparently stimulated to rapid division and reproduction, and the disease process is made worse rather than better. Large lymphocytes and cells of the monocyte group are but little affected by therapeutic doses of roentgen rays.

After appropriate treatment with effective doses of roentgen rays or radium, there is a progressive morphologic maturation of the red and white blood cells,

well seen in the polymorphonuclear series. On the day after the treatment there is usually an increase in the white blood cell count and the number increases until the cells reach the stage of active leucocyte movement, when they leave the blood stream by wandering out of the vessels through the mucous membranes of the mouth and probably the stomach.¹⁰ Under similar conditions the lymphocytes are discharged through the mucous membrane lower down in the intestine.¹¹ The increase in maturation of the cells can be noted by the increase in number of polymorphonuclear leucocytes containing nuclei with many lobes instead of the single round nucleus of the metamyelocyte. It has been shown¹² that there is a progressive increase in the morphologic evidence of age under these conditions. It is characterized by a marked "shift to the right" in the Aineh count.¹³ There is a demonstrable maturation of irradiated cells as shown by the increase in neutral red staining granules and the decrease in Janus green granules after irradiation, the former granules being characteristic of old cells, the latter, of young ones.¹⁴

The roentgen rays, then, stimulate certain cells in some stages to grow old and die of senility or be eliminated, while in other stages the cells are made to grow more rapidly. The first effect is desirable, the second, undesirable. Therefore, since both of these changes take place every time a patient is exposed to the radiation, it is advisable to use this type of therapy as few times as possible, especially as each treatment becomes less effective. It should be used only when (a) there are pressure symptoms from enlarged glands or spleen, (b) when there is a high white blood cell count associated with a high basal metabolic rate, (c) when the progressive invasion of the bone marrow by the leucemic tissue is crowding out the erythroblastic tissue and is resulting in a continuous fall in the red blood cell count and hemoglobin content. There is no indication for a routine irradiation at certain intervals, as the radiation does not cure the disease but has a specific effect on the immature cells in the body at that given time. Treatment may be applied over any part of the body, preferably a vascular area, such as the spleen or mediastinum. Roentgen ray or radon treatment is contraindicated when the majority of the leucocytes are in the "blast" stage, as in the acute leucemias, or the myeloblastic, lymphoblastic or monocytoblastic stage of the chronic leucemias. This is the so-called refractory type or stage of the disease.^{15, 16}

Blood transfusion has some effect in alleviating some of the symptoms, especially when there is a marked degree of anemia. Frequently there is a temporary decrease in the white blood cell count after a transfusion. Iron, in simple forms, is of some value in chronic lymphatic leucemia, and whole liver is also of some use. Liver or liver extract appears to be of little value in the chronic myelogenous form.

Lugol's solution has a definite effect, especially in chronic lymphatic leucemia, in relieving some of the symptoms associated with a high basal metabolic rate.¹⁷

Because of the low resistance to infection and tendency to bleed in leucemia, special preparations must be made before any operative procedures are carried out. It is advisable to give roentgen ray treatment until the white blood cell count is nearly normal, as the leucocytes prevent the formation of an efficient

clot in myelogenous leucemia and the platelets are deficient in number in the myelophthisic anemia in the lymphatic and acute types of the disease. Routine removal of the teeth as foci of infection is contraindicated. Tonsillectomy has been of no great value in this disease and splenectomy has no marked effect in prolonging the life of the patient or stopping the disease.

At present all that can be hoped for from the treatments in use is the temporary restoration of the patient to a degree of efficiency which will enable him to live a useful life at least during part of his disease. The average life of the patient is about three and a half years, with fairly wide extremes which appear to be related to the constitution of the patient and not to the therapy.¹⁸⁻²²

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TREATMENT OF THE ANEMIAS^{*}

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THE most essential requirement in the treatment of an anemia is the accurate recognition of the particular variety of the condition which is present. This is absolutely necessary because certain therapeutic measures which are very efficient in the treatment of some anemias are entirely useless in others. For example, liver extract and ventriculin are highly potent in pernicious anemia but are ineffective in the chronic secondary anemias. On the other hand, large doses of iron produce splendid results in some varieties of the latter condition, but they are without effect in pernicious anemia. Splenectomy is a specific cure in chronic hemolytic jaundice but is contraindicated in the myelophthisic anemia which is associated with Hodgkin's disease, and which is improved by the therapeutic application of the roentgen rays. The anemia of myxedema yields readily to treatment with desiccated thyroid gland but is uninfluenced by liver or desiccated stomach which is sometimes given because the patient is thought to have pernicious anemia.

THE TREATMENT OF CHRONIC SECONDARY ANEMIA

The term secondary anemia was originally introduced to include those anemias which were due to recognizable causes, in contrast to the primary anemias of unknown etiology. For some years, however, it has been customary to include in this group also those anemias with a low color index, despite the fact that their cause was unknown. This appears to be a logical grouping and, therefore, the anemias which will be included under the head of "chronic secondary anemia" in this article are those due to known causes or those which have a low color index, or those meeting both requirements.

It is difficult to estimate accurately the frequency with which the various anemias are encountered in the practice of medicine but undoubtedly chronic secondary anemia has the greatest incidence. This is true because there are so many different causes responsible for the condition. It may be associated with chronic blood loss due to uterine disorders, hemorrhoids, bleeding peptic ulcer, neoplasms, improper diet, infections, and a large number of chronic long continued diseases.

The essential requirement in the treatment of secondary anemias is to remove or control the underlying cause if this is possible. Until this is accomplished, it is too much to expect that various measures employed to overcome the anemia will produce their full effect. The first step, therefore, in treatment consists in subjecting each patient to a thorough study in an effort to discover the cause of the anemia and removing it, if possible. If the anemia is secondary to

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bleeding, every effort should be made to check this. Various infections, if present, should be eliminated but caution should be used in ascribing the anemia to various foci, such as teeth and tonsils. There should be reasonable assurance that they play a significant causative rôle in the production of the anemia before they are removed. Extraction of all the teeth has not only failed to cure the anemia in many instances, but has actually done harm on account of the transient resultant mouth infection and the subsequent inability to ingest a normal diet for a considerable period of time. In the anemias associated with Hodgkin's disease and the leucemias, improvement can only result from irradiation with radium or the roentgen rays.

In many types of secondary anemia it is beneficial to administer *large* doses of iron and in at least one type it has a specific action. This variety is the idiopathic microcytic or hypochromic anemia which appears to be increasing in incidence during the past few years. It occurs in women of middle age and is characterized by asthenia, vague gastrointestinal complaints, decreased resistance to infection, and a favorable response to iron medication. This condition has not received the emphasis to which it is entitled, as it occurs rather frequently. To overlook the condition and omit the proper treatment is regrettable for the response to iron is very striking. It resembles chlorosis in some respects, but differs in the age incidence, as it occurs most frequently in women between the ages of thirty-five and forty-five years. The etiology is unknown although probably the cause is concerned with the metabolism of iron, and one view regards it as an iron starvation resulting from defective absorption.

The important consideration when administering iron is not the nature of the preparation, but the size of the dose. As Witts¹ very aptly states, "Few facts in medicine are so unanimously believed by the expert, so strongly backed by evidence, and so little known to the profession, as the absolute necessity of very large doses of iron in the treatment of these chronic anemias." Poor results in the past have been due in many instances to an inadequate dosage. A convenient method of administering iron is in the form of ferric ammonium citrate which may be given in a 50 per cent solution in doses amounting to 4 to 6 grams (60 to 90 grains) daily, but probably the simplest and most satisfactory form is reduced iron given in 0.5 gram ($7\frac{1}{2}$ grain) capsules three times daily. All of these dosages are very much larger than are advised in most textbooks or in the pharmacopeia, but satisfactory results will not be obtained unless these amounts are used. Contrary to the usual belief, these large doses are well tolerated and rarely cause digestive disturbances.

The dietary habits should be investigated in all patients with a secondary anemia when the cause is not obvious, for there is considerable evidence that the ingestion of an obviously abnormal diet over a long period of time may cause a definite anemia. This occurs principally in three types of patients, (1) those who have difficulty in consuming food on account of cardiospasm or other mechanical causes, (2) those who consume a most abnormal diet as the result of personal whims, and (3) patients with hypertension or nephritis who have existed on a diet, usually exceedingly low in protein, for a long period. The cure of the anemia can only follow the taking of a proper diet which should be prescribed for the patient.

THE TREATMENT OF PERNICIOUS ANEMIA

Before the year 1926 the treatment of pernicious anemia was highly unsatisfactory as there was no form of therapy, with the possible exception of blood transfusions, which would prolong the life of a patient with this disease. With the introduction of liver as a form of therapy by Minot and Murphy² in the year just mentioned, liver extract in 1927 by Minot and Cohn et al.,³ and ventriculin by Sturgis and Isaacs in 1929,⁴ the entire outlook concerning the disease has been altered. The experience of a fairly large number of observers has demonstrated that the anemia of pernicious anemia can be made to disappear following the proper use of any one of the three forms of treatment in uncomplicated cases. The essential part of the treatment is to administer an adequate amount of liver, either cooked or uncooked, a potent liver extract or ventriculin (desiccated, defatted hog stomach) until the blood returns to normal. When this has been attained, a sufficient quantity of one of these substances must be given regularly in order to maintain the blood within normal limits. The average initial dosage of any one of these three forms of treatment which will cause the red blood cell count to rise to normal at the rate of about 400,000 red blood cells per cubic millimeter per week, is as follows: Liver, $\frac{1}{2}$ pound daily, liver extract, 5 to 6 vials daily (each vial representing 100 grams of liver), or ventriculin 10 grams daily for each million deficit in the total red blood cell count. The liver extract may be given either dissolved or suspended in water, tomato juice, ginger ale, or grape juice.

The effect of the treatment is prompt and striking. Within three to six days the patient shows evidence of improvement as indicated by an increase in appetite, a disappearance of gastric symptoms, such as nausea and vomiting, a gain in strength, and a fall in the pulse rate and body temperature to normal. The improvement is progressive and often after a few days to a week, a patient who has been confined to bed on account of weakness is able to sit up or walk about. Patients with uncomplicated pernicious anemia usually improve very rapidly and are able to resume a normal life within six to eight weeks after the beginning of the treatment.

The earliest change in the blood is not an increase in the number of red blood cells but an increase in the number of reticulocytes, or immature red blood cells. If the changes in cells are charted in the form of a curve, a definite rise in their number will be observed to begin between the third and sixth day of treatment. Following this there is a rapid increase until a peak of the curve is reached between the seventh and ninth day. There is then a gradual decline to about 1 per cent on approximately the fifteenth day of treatment. The height of the peak of the curve has an inverse relationship to the initial level of the red blood cell count just prior to treatment. For example, if the red blood cell count is one million, the height of the peak will be about 34 per cent, if the red blood cell count is two million, the height will be about 14 per cent, if the red count is three million or over, there will be slight if any increase in the number of reticulocytes following treatment. The red blood cells may not change during the first two weeks of treatment, or there may even be a decrease in their numbers. During subsequent weeks of treatment, however, the red blood cell count

may use a million per cubic millimeter per week or more, so that the average increase, from the beginning of treatment until the blood is normal, is about 400,000 per cubic centimeter per week

In some instances it has been reported that liver extract or ventriculin has failed to produce satisfactory results when used in the treatment of pernicious anemia. Careful investigation has usually disclosed one of the following reasons for this conclusion

1 The diagnosis of pernicious anemia has been incorrect. The patient may have been suffering from one of the forms of chronic secondary anemia, aleucemic leucemia, myxedema or some other type of anemia. In my experience, liver or stomach therapy is effective only in the treatment of the Addisonian type of pernicious anemia, the anemia secondary to *Dibothyocephalus latus* infestation, the so called "pernicious anemia of pregnancy" and some types of anemia associated with spinae

2 An acute infection has been present which inhibits to some extent the efficiency of liver or stomach therapy. This may be any type of infection, such as a cystitis, pyelitis, erysipelas, pneumonia, acute tonsillitis, or any variety of infection which is associated with a febrile reaction. In all patients who have such a complication the dosage of the preparation used should be increased 100 per cent as long as the infection is active

3 The liver extract or preparation of stomach has been of weak potency or completely inert. The only certain way to avert this possibility is to employ material which has been clinically tested and certified as having full potency. In very rare instances a patient is observed whose red blood cell count may reach a level of 2.5 to 3 million per cubic millimeter where it remains, for some unknown reason, despite large doses of liver or stomach by mouth. These patients may be treated with good results by intramuscular or intravenous injections of liver extracts which have been specially prepared for this purpose. Some such preparations when given intravenously may be followed by profound reactions, as evidenced by chills, fever, and hypertension, but undoubtedly this method of therapy is one of great promise if the cause of the severe reactions is eliminated

In order to obtain satisfactory results, all patients with pernicious anemia should remain under observation until their red blood cell count reaches normal, although after their red count is three million per cubic millimeter or more, they need be seen at less frequent intervals. During this period of observation the patient should have the red cells counted at frequent intervals, as the level of the red count is the best single criterion of the effectiveness of the treatment. If this remains low after the patient has received the average dosage of the preparation for a reasonable length of time, the amount should be increased. The patient should be warned that a relapse will follow in six weeks to several months after therapy is discontinued

Experience has shown that only liver or ventriculin is necessary in order to bring the blood of patients with pernicious anemia to normal. Such additional forms of therapy as iron, arsenic, and dilute hydrochloric acid appear to be entirely unnecessary. Some patients with pernicious anemia when first seen are extremely ill and have a profound anemia. All such patients should have their blood grouped for transfusion and arrangements made to have a suitable

donor at hand. Transfusions may be advisable in order to support the patient until the therapeutic effect of liver or stomach is apparent.

Although this article is primarily concerned with the treatment of the anemia of pernicious anemia, it seems proper to discuss briefly the effect of the treatment on the spinal cord complications which are not uncommon. The results attained in the treatment of this condition are far less satisfactory than those directed toward the relief of the anemia. Usually the minor neurologic manifestations, such as numbness and tingling of the extremities, completely disappear or become much less as the blood condition improves. Other symptoms, as a spastic paraplegia or loss of the sense of position of the extremities, which indicate a more serious lesion of the spinal cord, may improve but more frequently are arrested and occasionally advance despite all therapeutic efforts.

TRANSFUSIONS

Blood transfusion, either of citrated or noncitrated blood, is useful in the treatment of various types of anemia and should be considered under the following circumstances:

- 1 As an emergency measure to sustain life until bleeding can be checked or other effective measures introduced to combat the anemia. A patient at the point of death from traumatic hemorrhage may survive only as the result of a transfusion while appropriate surgical procedures are performed. As previously mentioned, patients who are critically ill with pernicious anemia may be temporarily improved by one or more transfusions and thereby survive until the permanent beneficial effects of liver or stomach become apparent.

- 2 As a method of treating the excessive bleeding in hemophilia and thrombocytopenic purpura. In both conditions there may be a severe associated anemia and in both, the bleeding is controlled by blood transfusions. Patients with hemophilia can be carried through major operations by repeated blood transfusions, given before and after the operation. Even such minor procedures as the extraction of a tooth in such patients should be preceded by blood transfusions and the operation not performed until the coagulation time reaches normal.

- 3 Patients with an anemia which is curable by surgical means should not be operated upon until repeated transfusions have caused the blood to approach normal limits. Splenectomy in chronic hemolytic jaundice, Banti's disease, and chronic idiopathic thrombocytopenic purpura should not be performed without one or more transfusions preceding the operation if an anemia is present.

- 4 Some believe that one or more transfusions should be advised in the anemia of chronic hemorrhage after the bleeding has been checked in order to expedite the return of the blood to normal. While this may be indicated occasionally, it should not be done routinely as the regeneration of blood is usually at a satisfactory rate when assisted only by a nutritious diet and large doses of iron.

SPLENECTOMY

This condition has been advised as a therapeutic measure in the anemia of pernicious anemia, the anemia associated with the leucemias, chronic hemolytic jaundice, splenic anemia, and chronic idiopathic thrombocytopenic purpura.

(purpura hemorrhagica) Observations have shown that the operation is no longer indicated in pernicious anemia and the leucemias, as evidence indicates that neither of these conditions is benefited by the operation. Furthermore, the use of stomach or liver therapy produces excellent results in pernicious anemia and the roentgen ray is the most effective treatment available at present for the treatment of the leucemias.

Chronic hemolytic jaundice is apparently curable by splenectomy which is indicated if the patient's symptoms are of such intensity as to warrant a major operation for their relief. Within a short time after the operation, the jaundice disappears, the blood returns to normal, and all symptoms subside. In many patients with this disease the symptoms are very mild and a major operation is not justifiable. A conclusion concerning the advisability of splenectomy must be decided in each individual case after a careful consideration of the patient's history, physical examination, and blood studies.

Splenectomy is indicated in splenic anemia (Banti's disease), especially during the preascitic stage when cirrhotic changes have not occurred in the liver, as this is the only form of therapy which is effective. Some consider that it should be performed even when there is definite evidence of an associated cirrhosis of the liver. Before such radical treatment is carried out, however, there should be every assurance that the diagnosis is correct. The syndrome may be closely simulated by Hodgkin's disease, with an enlarged spleen and an absence of peripheral lymph gland enlargement and by syphilis with splenomegaly. Preceding the operation, all such patients should be given a several weeks' course with large doses of potassium iodide as a therapeutic test for syphilis. If no change occurs, several applications of the roentgen ray should be given over the spleen. If the splenomegaly is due to Hodgkin's disease, there should be a prompt decrease in the size of that organ.

Splenectomy must be considered in all patients with chronic primary purpura hemorrhagica (chronic idiopathic thrombocytopenic purpura), provided the symptoms are severe enough, and if they are not controlled by repeated transfusions. The results in the chronic cases are satisfactory but the operation is contraindicated in the acute type on account of the high mortality.

MISCELLANEOUS FORMS OF THERAPY

Various preparations of arsenic have been recommended in the treatment of several types of blood diseases. For many years it was used in the form of Fowler's solution in the treatment of pernicious anemia, and good results were reported. Although it may cause an improvement in the patient's condition, the beneficial effects cannot be compared with those obtained by stomach or liver therapy. Nor is there convincing evidence that arsenic alone is of value in the treatment of the secondary anemias, or that a combination of arsenic and iron is any more effective than iron alone.

Spleen marrow has been advocated as an effective therapeutic agent in the secondary anemias. It has proved ineffective in my experience, and the reported results are not as satisfactory as those observed following efficient iron therapy.

Copper has been employed to some extent in the past two years in the treatment of the secondary anemias, but the clinical results, at least in adults,

do not indicate that it has a beneficial effect. This form of therapy was introduced as a result of convincing experimental evidence that minute amounts of copper cured a nutritional type of anemia in rats.

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II STUDIES ON PATIENTS WITH PERNICIOUS ANEMIA TREATED WITH MASSIVE DOSES OF LIVER EXTRACT

EFFECTS ON RETICULOCYTES, RED CELLS, HEMOGLOBIN AND WHITE CELLS*

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THE clinical and hematologic effects of the administration of massive doses of liver extract have been reported on by Riddle and Sturgis,¹ and more recently by Connery and Goldwater.² Two of the four cases included in the series of the latter observers received transfusions in addition to the massive doses of liver extract.

It is the purpose of this paper to present data on a series of fifteen cases of pernicious anemia treated with single massive doses of liver extract, with or without transfusion, or with repeated massive doses, with or without transfusion. These data are considered under the following headings: (a) effects on reticulocytes, (b) effects on red cells and hemoglobin, (c) effects on the white cells, (d) effects on the blood sugar, and (e) effects on the blood pressure.

MATERIALS AND METHODS

Of the fifteen cases of pernicious anemia included in this study, ten were males and five were females. Eight were admitted to the hospital in their first relapse, four in their second relapse, and three in their third relapse. Five of the patients gave a history of having previously taken some form of liver therapy, whereas no history of previous liver therapy was obtained from the other ten. Six patients were admitted with red cell counts below one million, six more had counts between one and two million, two had counts between two and three million, and one had a count greater than three million. Four of the patients exhibited signs of well established subacute combined sclerosis. The other eleven showed only slight neurologic involvement. In eight of the patients, one or more of the following complications were present on admission: acute or chronic

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bronchitis subacute cholecystitis, subacute or chronic sinusitis, chronic cystitis associated with hypertrophy of the prostate and residual urine, sloughing varicose ulcers with cellulitis, dental apical abscesses. In six of the patients the severity of the illness was of a mild degree, in three the severity was of a moderate degree, while the remaining six were critically ill. All of the cases included in this study were patients on the wards of the Third (New York University) Medical Division of Bellevue Hospital.

All apparatus used was standardized. Determinations on each patient were made by the same observers, using the same apparatus throughout the period of study. Where the condition of the patient permitted a control period of sufficient length was run to establish the range of the reticulocytes. In those cases in which it seemed that the life of the patient might be jeopardized if immediate treatment were not given, no control period was run. This fact, however, does not invalidate the results in these cases, since the time of beginning reticulocyte response, and the character of the reticulocyte curves were entirely comparable to those seen in the patients in whom there had been a control period. Daily reticulocyte determinations were made during the period of increased peripheral reticulocyte activity. Red cell counts, hemoglobin determinations, and white cell counts were made bi- or tri-weekly, and in some instances even more frequently. The "hemogram" was studied at least bi-weekly and in many cases as often as five times weekly, usually during the period of increased peripheral reticulocyte activity. The classification of Schilling³ was used, with a slight modification in the nomenclature. Blood pressure readings were made by the auscultatory method, using a mercury manometer. Readings were made at sufficiently short intervals so that any significant trend in the curves would be observed.

In estimating the blood sugar content, the method of Folin and Wu was used. Samples of blood were collected under standardized conditions at half hour intervals over a period of two hours. Transfusion was used only in those cases in which it seemed that the life of the patient would be endangered unless this measure was employed. While valuable information may have been adduced by the incidental use of transfusion, this procedure was not included as one of the objects of the study. The Lindemann method was used, and the amounts given were 300 c.c. to 650 c.c. per transfusion.

The liver extract* used was a commercial preparation of fraction G of Cohn, Minot et al.⁴ One vial of the extract represents the material obtained from 100 grams of whole mammalian liver. The amount of a single dose varied from thirty to fifty vials. The extract was dissolved in from 200 c.c. to 300 c.c. of tap water, milk or buttermilk, and was administered as follows. Nine cases (Cases 1, 2, 3, 4, 6, 8, 9, 11, 15) received the extract into the stomach by Rehfuss tube. Vomiting^{1, 2} occurred in Cases 1, 2, 4, 6, 8, 11, and 15. Four cases (Cases 7, 10, 11, 14) received the extract through a gastric lavage tube. Vomiting occurred in Case 10 only. Cases 5, 12, and 13 drank the extract in iced buttermilk. Vomiting occurred only in Case 5. It may be stated here that there seemed to be no relationship between the size of the dose, the mode of administration, the time of vomiting, the amount vomited, and the subsequent progress of the patient.

*Prepared by the Lederle Laboratories

RESULTS

Reticulocytes—Nine cases (Cases 1 to 9, Table I) were treated with single massive doses of liver extract without transfusion. Inasmuch as reticulocyte determinations were made at intervals of twenty-four hours, a beginning response noted as occurring on the second day actually took place between twenty-four and forty-eight hours, a response on the third day actually took place between forty-eight and seventy-two hours. Likewise, a peak noted as occurring on the fourth day actually took place between seventy-two and ninety-six hours, and so on.

TABLE I

CASE	DOSE	NO OF DAYS TO BEGINNING RESPONSE	NO OF DAYS TO PEAK	HEIGHT OF PEAK	NO OF DAYS OF I P R A	ESTIMATED RETICULO CYTE CON CENTRATION	CALCULATED RETICULO CYTE CON CENTRATION
1	50	*	*	*	*	*	*
2	50	3	7	26.0%	29	0.40	0.49
3	30	3	6	32.1%	9	0.37	0.57
4	50	3	6	12.5%	**		
5	30	2	6	24.8%	21	0.43	0.47
6	50	***	***	***	***	***	***
7	50	3	4	32.0%	13	0.37	0.57
8	50	4	6	12.0%	10	0.20	0.43
9	48	2	6	22.5%	11	0.52	0.37

*No response. Red blood cells at 3 million level. Previous reticulocyte peak.

**Left hospital at own request while reticulocytes were on the increase.

***No response. Red blood cells 3.5 million.

I P R A = Increased Peripheral Reticulocyte Activity.

Formula for Estimated Reticulocyte Concentration in millions = $E p^* = \frac{E o^*}{1 - r}$

Formula for Calculated Reticulocyte Concentration in millions = $E r = 0.73 - 0.2 E o$.

$E p^*$ = Observed concentration of reticulocytes at peak of rise.

$E o^*$

$\frac{E o^*}{1 - r}$ = Estimated concentration of reticulocytes at peak of rise.

$E r$ = Calculated concentration of reticulocytes at peak of rise.

$E o$ = Original level of erythrocytes.

$E p$ = Erythrocytes concentration at height of reticulocyte peak.

r = Reticulocytes in per cent.

There seemed to be no relationship between the size of the dose, and the number of days to beginning reticulocyte response, the number of days to reticulocyte peak, the height of the reticulocyte peak, or the number of days of increased peripheral reticulocyte activity.

No reticulocyte response was noted in Cases 1 and 6. Failure to induce increased reticulocyte activity by this form of treatment in Case 1 could be explained by two facts: (a) the level of the red cells at the time the massive dose was given was above three million, (b) the reticulocytes were increased above the normal at the time of admission (9 per cent) and rose to 17.4 per cent before

treatment was given (see Chart 1, Curve 1) In Case 6 the level of the red cells at the time treatment was given was 3.6 millions⁵ (Chart 1, Curve 6)

The administration of 50 vials of liver extract to Cases 4 and 8 induced reticulocyte peaks of no great magnitude, a fact which warrants explanation In the former, Case 4, the presence of subacute cholecystitis quite likely depressed the magnitude of the reticulocyte response, although it must be pointed out that the patient left the hospital at his own request at a time when the reticulocytes were on the increase In the latter, Case 8, the presence of chronic bronchitis and several abscessed teeth may have been responsible for the low level reached by the reticulocytes at their peak Three of the cases (Cases 2, 5, and 9) showed a satisfactory agreement between the estimated and calculated reticulocyte concentrations⁶ In three cases (Cases 3, 7, and 8) this agreement was not present

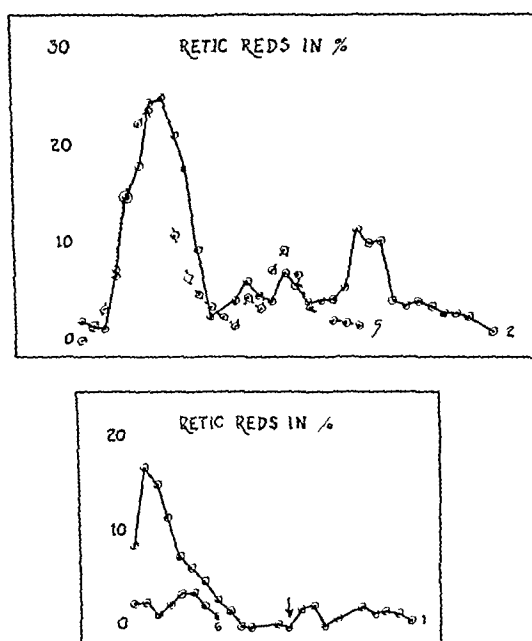


Chart 1—Treatment given on 0 day in all cases except Case 1 where day of treatment is indicated by arrow. Curve numbers coincide with case numbers. Each division on abscissa represents one day.

Reticulocyte concentrations could not be calculated in Cases 1, 4, and 6 due to incompleteness of data. In Case 7 the lack of agreement between the estimated and calculated reticulocyte concentrations was probably due to the presence of a purulent sinusitis and numerous apical abscesses. The factors responsible for depressing the magnitude of the reticulocyte response in Case 8 may also serve to explain the lack of agreement between calculated and estimated reticulocyte concentrations in this case.

Secondary reticulocyte peaks have been observed quite frequently in patients who have been treated with suboptimal amounts of liver or a potent extract in daily doses and later with optimal amounts. The same phenomenon may be seen when the administration of a weak liver preparation is followed by the use of optimal amounts of a potent preparation.⁶ Two of the cases (Cases 2 and 5),

treated with single massive doses of liver extract showed secondary peaks in their reticulocyte curves (Chart 1, Curves 2 and 5). The conditions mentioned above did not prevail in these cases, so that the significance of the secondary peaks remains a matter for speculation. These two cases were at the extremes of the scale of dosage, receiving 50 vials and 30 vials respectively, and of the entire series ran the most favorable courses, both hematologically and clinically.

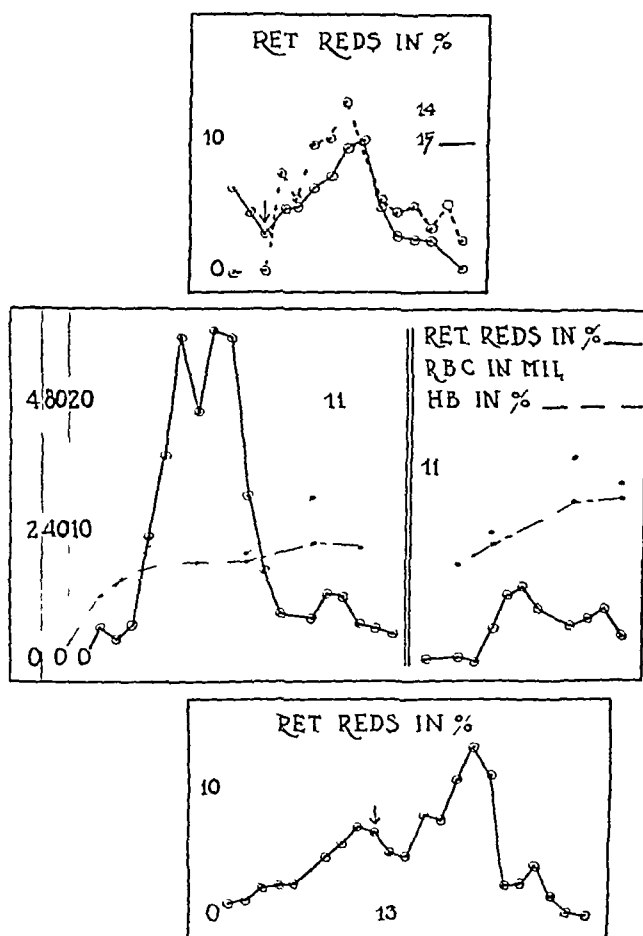


Chart 2—Treatment given on 0 day except where indicated by arrows in Curves 13 and 15. In the latter the first dose was given on 0 day and the second is indicated. Curve numbers coincide with case numbers. Each division on abscissa represents one day. In Case 11 the double vertical bar indicates an interval of twenty one days at the end of which time a second massive dose was given. In Curve 13 time from 0 to arrow represents period on kidney extract.

Case 13 (Chart 2, Curve 13) had a previous period of hospitalization, during which he was treated with Marme Liver Extract^s in daily doses of 90 c c. A satisfactory remission had been induced, but after the patient left the hospital he at first took the extract irregularly and eventually ceased taking it altogether. About a year later he was readmitted to the hospital in a well established relapse. During the first ten days he was given a daily dose of a kidney extract derived from 800 grams of whole mammalian kidney^s. The technique used in preparing

^sPrepared by the White Laboratories of Newark, New Jersey.

this extract was that used in the manufacture of a commercial extract of fraction G¹. Chart 2, Curve 13 shows that the administration of the kidney extract induced a slight reticulocyte response. After ten days of this treatment, when it became apparent that little or no further increase in the reticulocytes could be expected, and that only slight clinical improvement had occurred, it was deemed advisable to discontinue this form of treatment. Accordingly, a single massive dose of 50 vials of liver extract was given. On the sixth day following, the reticulocytes reached a peak of 12.8 per cent. That the reticulocytes did not reach a higher level can probably be explained by one or both of the following facts: (a) a slight reticulocyte response had just been elicited by what were either suboptimal amounts of a potent extract or maximal amounts of a weak extract, (b) lobar pneumonia supervened while the reticulocytes were still on the increase.⁵

TABLE II

HEMOGLOBIN DETERMINATIONS BY KLETT-NEWCOMER METHOD, 100% = 17 GRAMS HEMOGLOBIN PER 100 C.C. BLOOD

IN SOME INSTANCES THE HEMOGLOBIN REACHED A HIGHER LEVEL THAN THAT OF THE DAY WHEN THE RED BLOOD CELLS WERE AT THEIR MAXIMUM

CASE	EXTRACT		TRANSFUSION		INITIAL LEVEL		LEVEL FOLLOWING TRANSFUSION		MAXIMUM		TOTAL INCREASE*		NO. OF DAYS TO MAXIMUM
	DOSE IN VIALS	DATE	AMOUNT	DATE	RBC IN MIL	HB GR	RBC IN MIL	HB GR	RBC IN MIL	HB GR	RBC IN MIL	HB GR	
10	50	1/27/31	500 c.c.	1/27/31	0.53	2.1	1.25	5.0	2.6	8.6	1.35	3.6	21
11	50	10/14/30	500 c.c.	10/14/30	0.99	3.6	1.56	4.9	2.78	8.0	1.22	3.1	23
12	30	4/18/31	500 c.c.	4/18/31	0.70	3.2	1.40	6.5	2.65	7.4	1.2	0.9	10***
13	50	4/16/31	500 c.c.	4/27/31**									
14	42	8/ 1/30	650 c.c.	8/ 2/30									
	42	8/ 2/30	500 c.c.	8/ 5/30	0.65	1.6	2.4	6.0	4.8	11.8	2.4	5.8	51

*Counting from after transfusion

**Transfused because of pneumonia

***Erysipelas developed

Transfusion in conjunction with single or repeated massive doses of liver extract was used in a series of five cases (Cases 10 to 14, Table II). Of these, two cases (Cases 10 and 12), received a single transfusion of 500 c.c. of whole blood and a single massive dose of 50 vials and 30 vials of liver extract respectively. The response of the reticulocytes in these two cases was quite similar to that seen in comparable cases treated with single massive doses without transfusion.^{2, 10} The reticulocyte curves of these two cases were almost exact duplicates of the first part of Curve 11, Chart 2, of which represents the curve of the reticulocytes following a single massive dose, plus transfusion. This case is treated separately because a second massive dose was given later, as will be discussed below. The similarity of this curve to Curves 2 and 5, Chart 1, those of cases treated with single massive doses without transfusion, is quite striking. The first part of Curve 11, Chart 2, already mentioned, shows the reticulo-

cyte response following a single massive dose and a transfusion, in Case 11. The second part of Curve 11, Chart 2 shows the reticulocyte response following a second massive dose, which was given when it had become apparent that no further increase in red cells or hemoglobin could be expected following the first massive dose of liver extract. Although the size of the second dose was the same as that of the original dose, and the level of the red cells was practically no higher than it was at the time the first dose was given, the reticulocyte response following the second dose was of a much smaller magnitude than that of the original reticulocyte response. The explanation for this probably lies in the fact that a major period of increased peripheral reticulocyte activity had just been completed.⁶

Case 14 received two doses of 42 vials each and transfusions of 650 cc and 500 cc respectively, all within a period of four days. The response of the reticulocytes to this treatment is shown on Chart 2, Curve 14. Two features are worthy of comment. First, the beginning reticulocyte response did not occur any sooner than that in cases receiving smaller doses. Second, the magnitude of the reticulocyte response was not great. Both of these occurred in spite of the fact that the patient received in less than twenty-four hours the extract derived from 8,400 grams of whole mammalian liver. Just what effect the transfusions had on the reticulocyte response is a matter for speculation.^{9, 10} Curve 15, Chart 2 represents the reticulocyte curve of Case 15, who received two doses of 50 vials each within a period of forty-eight hours. It is to be noted that Curves 14 and 15 are quite similar. The presence of a low grade, chronic cystitis as associated with an hypertrophied prostate and residual urine probably explains

TABLE III

HEMOGLOBIN DETERMINATIONS BY KLETT-NEWMAN METHOD, 100% = 17 GRAMS HEMOGLOBIN PER 100 CC BLOOD

CASE	INITIAL LEVEL		MAXIMUM		TOTAL INCREASE		NO OF DAYS TO MAXIMUM
	RED BLOOD CELLS (IN MILLIONS)	HB (IN GRAMS)	RED BLOOD CELLS (IN MILLIONS)	HB (IN GRAMS)	RED BLOOD CELLS (IN MILLIONS)	HB (IN GRAMS)	
1	2.6	8.0	3.7	10.0	1.1	2.0	21
2	1.2	5.4	4.1	13.5	2.9	8.1	34
3	0.8	3.6	2.0	6.6	1.2	3.0	9
4*	1.5	5.0	1.6	5.0	0.1	0.0	6
5	1.3	4.5	3.7	11.4	2.4	6.9	51
6	3.6	11.8	4.0	11.5	0.4	0.3	16
7	0.8	3.4	2.7	9.3	1.9	5.9	32
8	1.5	6.4	1.9	5.7	0.4	0.7	16
9	1.8	5.4	4.4	13.5	2.6	8.1	35

*This patient left the hospital while the reticulocytes were on the increase. In some instances the hemoglobin reached a higher level than that of the day when the red blood cells were at their maximum.

the unsatisfactory reticulocyte response of Case 14. It is not entirely certain that the reticulocyte response in Case 15 (Chart 2, Curve 15) is unsatisfactory, in view of the fact that liver had been taken irregularly prior to the time of admission to the hospital.⁶ It seems likely, however, that the reticulocyte peak would have been higher had there not been present a low grade pyelitis.

Red Cells and Hemoglobin—Data concerning the quantitative changes in the red blood cells and hemoglobin are presented in Tables II and III. In general, the figures speak for themselves, but several points seem deserving of comment. In the nontransfused group (Table III), Cases 2, 5 and 9 will be seen to have had the most favorable red cell responses. These cases had an initial level of between one and two millions. Cases 4 and 8 had similar initial levels, but cannot be included in the generalization, the former because of incomplete data, the latter because of the presence of complications. Of the transfused group (Table II), Cases 10, 11, and 12 had their red cells raised to between one and

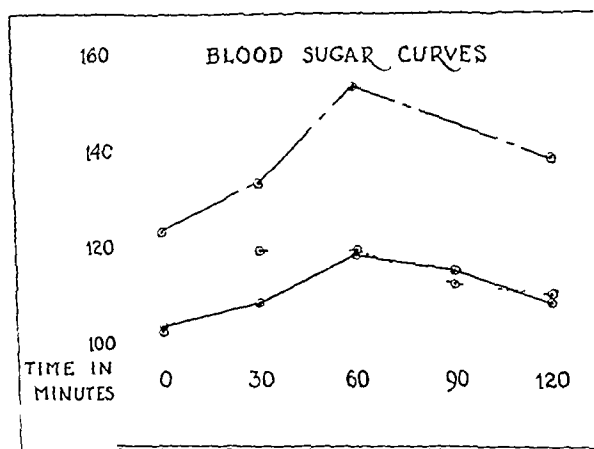


Chart 3—Blood sugar curves in three representative cases. Blood sugar in milligrams per 100 c.c. blood

two millions by transfusion. The increase in erythrocytes was not as great in these cases as that seen in a similar but nontransfused group, Cases 2, 5, and 9.

The lack of agreement between the increase of the red cells in these two groups becomes more striking when it is recalled that the original level of the transfused group was below one million. It may be that from the initial level of the red cells one cannot predict the ultimate level to be reached following a single massive dose, as can be done in the case of the reticulocytes.

As would be expected in those cases treated with a single massive dose without transfusion, the greatest increases in hemoglobin were observed in those cases that showed the greatest increases in red cells (Table III). In Case 4 no increase in hemoglobin was noted. Inasmuch as this patient left the hospital at a time when the reticulocytes were on the upward trend, there is no saying what the ultimate level of the red cells and hemoglobin might have been. In Cases 6 and 8 the apparent decreases in hemoglobin might be regarded as coming within the range of the normal fluctuations which are frequently observed in patients under treatment.

What has been said of the hemoglobin in the nontransfused group may also be said of the cases treated with single or repeated massive doses plus single or repeated transfusions, that is, those which showed the greatest increases in hemoglobin were those in which the greatest increases in red cells had occurred (Table II). In Case 12 the occurrence of erysipelas while the red cells and hemoglobin were still on the increase may explain the slight increase in hemoglobin.

White Cells—In Table IV are given the hemograms of seven cases. Five cases (Cases 2, 5, 7, 9, and 11) showed increased total leucocyte counts during the period of increased peripheral reticulocyte activity. Of these, one (Case 11) showed also a decided shift to the left. A shift to the left was also seen in Case 7,

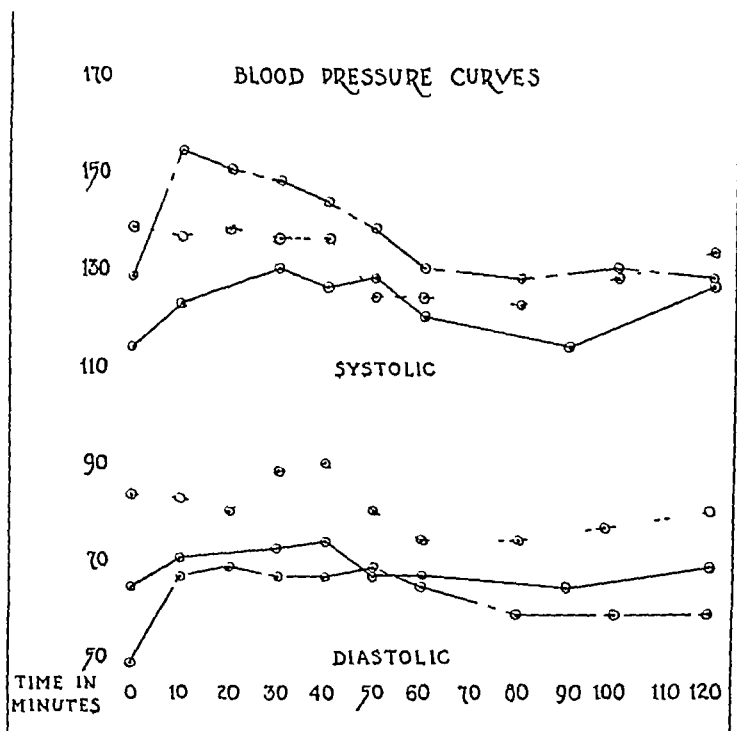


Chart 4—Blood pressure curves in three representative cases. Blood pressures in millimeters of mercury.

but not associated with any increase in total leucocyte count. Three cases (Cases 2, 5, and 9) showed an increase in polymorphonuclears with no shift to the left, but associated with a decrease in lymphocytes. One, Case 4, showed a decrease in polymorphonuclears, associated with an increase in lymphocytes and no change in the total count. Those cases that showed the greatest hematologic and clinical improvement following a single massive dose of liver extract were those that had the lowest total leucocyte counts before treatment was given (Table IV, Cases 2, 5, and 9). As already noted, these were also the cases that showed an increase in leucocytes associated with no shift to the left, but with an increase in polymorphonuclears and a decrease in lymphocytes. A distinct shift to the left was noted twice (Cases 7 and 11, Chart 4). These were not the cases that showed the greatest hematologic and clinical improvement in spite of the fact that a

shift to the left means either increased leucoblastic activity, or more rapid delivery of young white cells, or both, and, by implication, increased erythroblastic activity

Blood Sugar and Blood Pressure—In discussing dosage and administration, vomiting was noted as occurring in several of the cases. Although it seemed most likely that this could be explained on the basis of local action, in view of the work of Blotner and Murphy¹¹ and of Althausen, Keil and Burnett,¹² it seemed worthwhile to determine whether or not the gastrointestinal disturbances might be related to fall in blood sugar, or fall in blood pressure, or both. It is true that the liver fractions used by the above observers were not the same fractions

TABLE IV
TOTAL LEUCOCYTE COUNTS AND DIFFERENTIAL FORMULAE (HEMOGRAMS), BEFORE AND DURING RETICULOCYTE RESPONSE TOTAL LEUCOCYTES IN THOUSANDS DIFFERENTIAL FORMULAE IN PERCENTAGE

	BEFORE RETIC RESPONSE	AFTER RETIC RESPONSE	BEFORE RETIC RESPONSE	AFTER RETIC RESPONSE	BEFORE RETIC RESPONSE	AFTER RETIC RESPONSE	BEFORE RETIC RESPONSE	AFTER RETIC RESPONSE	BEFORE RETIC RESPONSE	AFTER RETIC RESPONSE	BEFORE RETIC RESPONSE	AFTER RETIC RESPONSE	BEFORE RETIC RESPONSE	AFTER RETIC RESPONSE
TOTAL	25	70	60	65	48	115	50	51	60	125	40	70	85	140
Myelocytes	0	0	0	0	0	0	0	12	0	0	0	0	0	26
Meta I (Young)	0	0	0	0	0	0	0	11	0	0	0	0	0	7
Meta II (Band)	1	1	2	0	2	2	3	9	0	9	2	2	4	4
Polymorpho nuclears	29	58	75	38	16	43	89	58	75	73	66	82	74	48
Lympho cytes	67	34	19	58	76	49	8	9	21	8	30	11	17	10
Monocytes	2	6	2	2	2	5	0	1	2	8	2	5	4	4
Eosinophiles	1	1	1	2	4	0	0	0	2	2	0	0	1	1
Basophiles	0	0	1	0	0	1	0	0	0	0	0	0	0	0
Plasma	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Case	2		4		5		7		8		9		11	

that are employed in the treatment of pernicious anemia, nevertheless, we know of no published reports of the effects on blood sugar¹³ and blood pressure of such large doses of liver extract as were used in our series. Chart 3 gives three representative blood sugar curves. It is quite apparent that no marked fall in blood sugar occurred. The curves of systolic and diastolic blood pressure in three representative cases are given in Chart 4. Here again it will be seen that no marked fall in blood pressure followed the administration of massive doses of liver extract.

SUMMARY

1. Fifteen cases of pernicious anemia were treated with single or repeated massive doses of liver extract, with or without transfusion.

2 Data are presented on the effects on reticulocytes, red cells hemoglobin white cells, blood sugar, and blood pressure

3 The reticulocytes responded promptly in the complicated as well as in the uncomplicated cases

4 Two cases showed no reticulocyte response, one because of a previous reticulocyte peak and the other because of the high level of the red cells at the time of treatment

5 The magnitude of the reticulocyte response seemed to depend on the initial level of the red cells and the presence or absence of complications

6 Within the limits of the dosage used, the size of the dose seemed to bear no relationship to the promptness of the reticulocyte response the height of the reticulocyte peak or the duration of increased reticulocyte activity

7 Transfusion seemed to have no effect on the promptness, magnitude or duration of the reticulocyte response

8 From the type of the reticulocyte response it was impossible to predict the subsequent course of the red cells

9 The increase in red cells bore no relationship to the size of the dose given nor to the original level of the red cells, but did seem to be influenced by the presence of complications

10 The course of the hemoglobin in general paralleled that of the red cells

11 Increase in white cells was noted in several cases, with or without shift to the left. Increases in some cases were associated with decrease in lymphocytes and increase in polynuclears. In one instance there occurred a shift to the left with no increase in the total number of leucocytes

12 The occurrence of a leucocytosis with or without shift to the left, could not be regarded as a favorable prognostic sign

13 Those cases that had the lowest initial leucocyte counts ran the most favorable course

14 The administration of 30 to 50 vials of liver extract was without significant effect on blood sugar or blood pressure

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A SIMPLE APPARATUS FOR THE TRANSFUSION OF BLOOD BY THE CITRATE METHOD*

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TRANSFUSION of blood by the direct method is a most satisfactory operation when performed by a well trained transfusion team. Often, however, the direct procedure is not applicable or possible under many conditions in which transfusion is indicated. Transfusion can never have the wider usefulness which its value as a therapeutic procedure deserves if only the direct method is employed.

The citrate method of transfusion has stood the test of time and is now accepted everywhere as a most satisfactory procedure. Poor technique is probably largely responsible for criticism of the indirect method. I am convinced that blood given properly by the citrate method is fully as valuable as that given by the direct method. The blood should be subjected to the least possible manipulation, and should be obtained and kept in a closed container. The proportion of citrate to blood should be maintained constantly at the optimum concentration (0.25 per cent). The apparatus described herewith fulfills the requirements mentioned above, and has been successfully used by a number of workers in several thousand transfusions.

The apparatus as set up for obtaining blood from the donor is illustrated in Fig. 1. A 500 to 700 cc wide-mouth bottle (a 1 pound glucose bottle is most satisfactory), fitted with a rubber stopper is employed. *A* is a stopcock with extension through the stopper to which a rubber tube touching the bottom of the bottle is attached. *B* is a metal sinker carrying a wire strainer. The citrate solution is measured in the barrel of a 20 cc Luer syringe. The solution may be run in drop by drop or 10 cc at a time. Mouth suction and a 13- to 15-gauge rustless steel needle are used in obtaining the blood. A trap of sterile absorbent cotton, *C*, is placed in the aspirating tube. A slight rotatory movement of the bottle as the blood is running in is all that is necessary for proper mixing. About 100 cc of blood should be obtained per minute.

The set up for giving the blood to the recipient is illustrated in Fig. 2. The syringe barrel has been removed and the inlet and aspirating tubes folded and out of the way. *D* is an automatic stopcock, connected to the stopcock *F*. The blood is withdrawn from the bottle and injected into the vein of the recipient by means of a 20 cc Luer syringe. If necessary, air may be expelled or salt solution may be drawn in through the side inlet, *E*. It is necessary, of course, to fill the tubes with blood before connecting them with the needle in the vein.

* From the Cleveland Clinic.

of the recipient I find it more convenient to attach the needle to a syringe, insert it into the vein and then connect the rubber tubing. The blood may be given rapidly or slowly. A few cubic centimeters of blood should be run in and the patient observed for a few minutes for signs of reaction before the remainder is given. Since there is always some drying of blood on the barrel of the syringe, it is usually necessary to use two or more syringes. These are easily exchanged after the stopcock, *F*, has been closed.

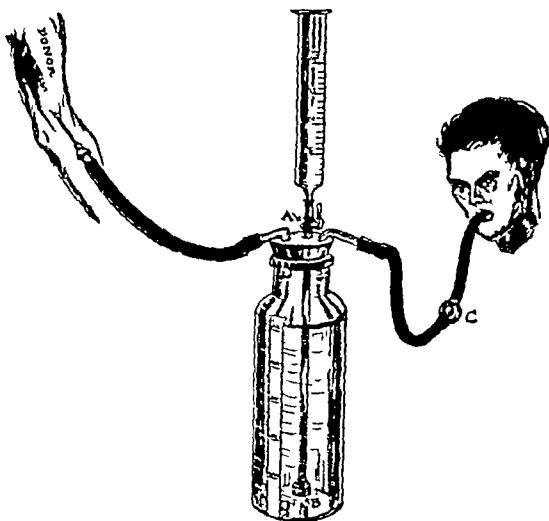


Fig 1—Drawing illustrating apparatus for obtaining blood from the donor

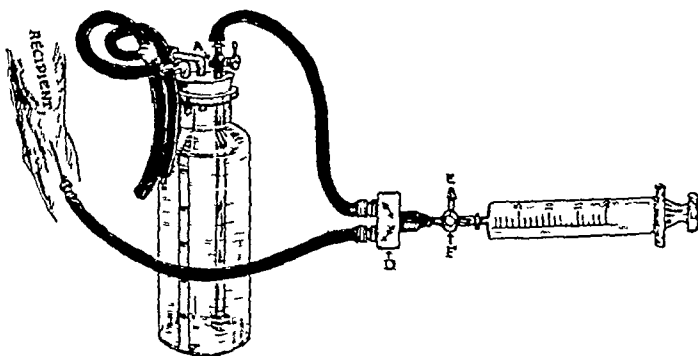


Fig 2—Drawing illustrating apparatus for transferring blood to the recipient

For proper functioning it is necessary, of course, that the apparatus be kept scrupulously clean. The automatic stopcock should always be taken apart after each transfusion and the valve stems and seats carefully cleaned. The component parts of the apparatus, including the rustless needles, are wrapped in towels and autoclaved so that the apparatus is always ready for instant use. I have found it convenient to have several sets with interchangeable parts made up and sterilized so that several transfusions a day are always prepared for.

I have prepared my own citrate solution. A 25 per cent solution of chemically pure sodium citrate in 0.85 per cent sodium chloride is put up in

hard glass ampules, which are autoclaved before they are sealed. Ten cubic centimeters of this solution is employed for 90 c.c. of blood which gives a final concentration of 0.25 per cent citrate in the citrated blood.

REACTIONS TO BLOOD TRANSFUSION

OBSERVATIONS FROM 2500 TRANSFUSIONS WITH A REVIEW OF THE LITERATURE

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ABOUT ten years ago, Karsner¹ stated that the problem of reaction to blood transfusion was by no means solved. Although this statement still holds true today, many of the previously unrecognized phenomena which were responsible for some of the reactions, have since been explained and are now under control. The causes of reactions to blood transfusion, however, are numerous and may be outlined as follows:

I Incompatibility between donor's and recipient's blood

This may be due to one of three causes:

(a) Errors in grouping the blood due to the following causes in order of importance

- 1 Poor technic
- 2 Use of low titered or contaminated test serum
- 3 Weak agglutinins or agglutinogens in the recipient's blood
- 4 Pseudoagglutination
- 5 Autoagglutination cold agglutination and subgroups
- 6 Anomalous or atypical agglutination
- 7 Contamination of recipient's blood by bacteria

(b) Indiscriminate use of the universal donor

(c) Immune isoantibodies and hemolysins

II The use of unclean apparatus

III The use of citrate solutions

IV Incipient coagulative changes in the transfused blood

V Allergic phenomena in the recipient

VI Systemic diseases in the recipient

VII Transmission of disease to recipient

INCOMPATIBILITY

Incompatibility between donor's and recipient's blood is due mainly to errors in grouping, because the tests are performed by internes who as a rule, do not stay on laboratory service long enough to acquire proper technic. Only the most experienced can entirely avoid the occurrence of such errors. Thus, Landeman² points out that posttransfusion chills occurred in 33 per cent of his cases when he allowed others to do the blood testing, as compared to 7 per cent when he performed the tests himself. Fisk³ justifiably urges that every hospital have at least one man on its staff who devotes himself exclusively to

this work Claimont⁵ even goes to the extent of stating that reactions will not occur if the blood groups are correctly determined Brooks⁶ and other writers have expressed similar opinions

One of the most common sources of error in grouping blood is the use of weak test sera This may be due to long standing at room temperature or to contamination by bacteria Dyke⁷ has shown that the serum may be considered sufficiently potent, only when it definitely agglutinates its specific isoagglutinin in dilutions of one to ten The tests must, of course, be performed on a 2 to 5 per cent cell suspension More recently, Coca⁸ found that test sera offered to the profession by reputable commercial biologic laboratories were below the required standard necessary for grouping purposes Bacterial contamination which, as a rule, diminishes the potency of test serum, may sometimes be the cause of an error in grouping, due to the agglutinating effect produced by the bacteria or their products An instance of this type is cited by Grove and Crum⁹ who found test serum unreliable because of contamination with mustard bacilli which, in the case cited, clumped cells of Group O, resulting in an erroneous determination of the group To avoid these errors, test sera should be checked frequently Weak agglutinogens or agglutinins in the recipient's blood especially in the case of infants¹⁰ may also lead to false group determinations Failure to recognize this fact has resulted in reactions¹¹ after repeated transfusion in children in whom the agglutinins developed or became active after the first transfusion For this reason, Astrowe¹² and others before him have recommended that reliance be placed only on the reaction of the corpuscles and not on the serum, when grouping children under two years of age In adults, however, the reactions due to such errors are not severe because the agglutinin or agglutininogen which is too weak to be detected in the grouping is also too weak to cause a marked reaction Thus, in a case previously reported by the present writers¹³ in which B blood was given eighteen times to an AB recipient whose agglutininogen A was weak and also in the case reported by Burnham¹⁴ in which A blood was given to a B recipient with weak agglutinin α , the reactions were only very mild or entirely absent The authors have encountered another similar case in which A blood was injected into a B recipient, apparently without any harmful reaction On careful reexamination of the blood of the donor by Dr A Wiener, it was found that the donor belonged to Group A_2 which is the less sensitive of the two subgroups of Group A This fact (as in the previous experience of the authors with the Group AB recipient) probably explains the absence of a reaction

Pseudoagglutination, which is characterized by a rapid sedimentation of the red blood cells, is a phenomenon frequently observed in pregnancy, menstruation, and in certain pathologic conditions (sepsis, tuberculosis, pneumonia, rheumatic fever) These false agglutinations occasionally lead to errors in blood grouping, but can be recognized microscopically by rouleaux formation It is not an antigen-antibody combination In contradistinction to agglutinins, the active principle in the serum is not absorbed by treating the serum with red cells¹⁵ A colloidal, mucoid, or gelatinous substance in the plasma may produce a phenomenon resembling pseudoagglutination An example of such a substance observed by the present writers is that of Wharton's jelly in the blood of the

umbilical cord. The same observation was made independently by Schott¹⁶. Pseudoagglutination can be readily avoided by diluting the serum or plasma with physiologic saline in the proportions of one to three. This subject is discussed at length by Shattock,¹⁷ Wiltshire,¹⁸ and Moirville¹⁹ and the characteristics of the phenomenon are excellently illustrated in a recent paper by Coca.²⁰

Autoagglutination²¹⁻²² is a phenomenon in which the red cells of a person are agglutinated by his own serum. This may occur normally at ice box temperature (cold agglutination). The clumping is due to a nonspecific agglutinin which acts on cells of all groups. In certain pathologic conditions, however, such as hemolytic icterus, paroxysmal hemoglobinuria, syphilis, and anemia, this phenomenon may occur even at room temperature. Occasionally this leads to error in grouping, because the tyro usually regards these cases as Group AB, although the blood which is being tested may in reality belong to any of the four groups. This reaction is recognized by examining a drop of the cell suspension without the addition of test sera, when it will be seen that there is spontaneous clumping of the cells. In these instances correct results can be obtained only by testing the *washed* red cells. *This should be done in all cases in which there is agglutination of the unwashed red blood cells with both A and B test sera.* In contrast to pseudoagglutination, cold agglutination resists slight or moderate dilution and the agglutinins are absorbable by the cells.²³⁻²⁴⁻²⁵ The action of these agglutinins is diminished by an increased temperature, and in most cases disappears at room temperature. It is never present at body temperature.

Some unexplained reactions may be due to the existence of differences in the blood which are not detectable by the ordinary methods of blood grouping. Thus, differences in the type of A cells have been demonstrated by von Dungen and Hirschfeld,²⁶ Coca and Klein²⁶ and others. These have been designated by Landsteiner and Levine³¹ as A¹ and A². Other differences in agglutinogens have recently been described by Landsteiner and Levine³² and referred to as M, N, and P. Normal isoagglutinins for M and N, however, have not been demonstrated. Therefore, these agglutinogens play no part in transfusion reactions.³³ It is also still to be determined whether the agglutinins specific to A¹, A² or P play an important rôle in reaction to transfusion. Landsteiner, Levine, and Janes have recently reported successful transfusion in 5 cases in which the patients who possessed the agglutinins under discussion, received blood of the same group. These investigators, however, feel that this matter requires further observation. It is therefore still essential to do mutual matching of donor's and recipient's blood in order to avoid any possible reactions which may ensue.

Down³⁴ claims that he has seen differences in the leucocytes of individuals of the same blood group and ascribes certain reactions to this phenomenon. These observations could not be confirmed by Rosenberger³⁵ working in Schiff's laboratory. Further studies on this problem are at present being made by Doan.³⁶

Anomalous or atypical agglutinins³⁷⁻³⁸⁻³⁹ which are rare, possess intermediate properties between cold agglutinins and typical isoagglutinins. Although they agglutinate red cells irrespective of the group (cells of their own group as well as those of Group O) these agglutinins are, as a rule, active only at room temperature of about 20° C, and very infrequently at 37° C. They therefore

rarely lead to errors in grouping. Experience, however, has shown that even this possibility must be kept in mind.

If the blood which is drawn from the recipient for grouping is not fresh (after twenty-four or more hours at room temperature), the red cells may become agglutinable by all human sera, thus causing a wrong determination of the group to which the patient belongs. Thomsen and Friedenreich³⁷ have shown this effect to be due to bacterial products. As a rule, however, suspensions of fresh blood are used for grouping, so that this is rarely a cause of difficulty. Lacy³⁸ also found that in cases of bacteremia the serum may contain agglutinating substances due to the infecting organism.

The changes in the blood group which Levine and Segall³⁹ claim to have found in patients who were subjected to prolonged etherization as well as those found by Eden⁴⁰ to be due to certain drugs (gumme, antipyrine) and by Benda and LeClere⁴¹ to be due to radio therapy, are probably all based upon erroneous observations, for it is now well known that the isoagglutminogen and isoagglutmin of the blood remain permanent after the second year of life. Landsteiner,⁴² the discoverer of isohemagglutination, is strongly of the opinion that a blood group, once established, never changes.

'Universal donors' are used as such by many on the grounds that the red cells of this group are not agglutinated by any human serum. Thus, Bimes⁴³ in a recent paper, advocates as a safe procedure the routine use of the 'universal donor.' Exception must be taken to this statement because of the not infrequent reports of alarming and even fatal reactions occurring as a result of such indiscriminate use of these donors. Unger⁴⁴ has reported several such observations and Cophee⁴⁵ mentions the occurrence of fatal accidents following such practice. The dangers involved in the routine use of the 'universal donor' have also been emphasized by the Inter-allied Surgical Congress in a recent report. In 1926, Freeman⁴⁶ reported the case of a universal donor who possessed an "alpha" agglutmin which was so powerful, that if he had acted as a donor for the other groups (A or AB), according to the author, the results would have been very serious. Similar dangers had been previously pointed out by Levine and Mabee.⁴⁷ Gray,⁴⁸ in a review on the results of five hundred transfusions in which donor and recipient were of the same group, found that there were only 10 per cent reactions as compared to 33 per cent of forty transfusions in which universal donors were used. Landsteiner is of the opinion that donors of the homologous group are preferable and also states that the clinical experience of Jones, Glynn, Kubanyi, Butka and Kratt support this view.

In the last decade there have been observed posttransfusion reactions which were apparently due to the development of isoantibodies in the plasma of persons who had previously received injections of compatible blood. In connection with this phenomenon, the following may be quoted from Landsteiner's chapter on the human blood groups:⁴⁹ "This consideration may have a bearing on the results of repeated transfusions in man, in view of the possible formation of immune isoantibodies in human beings, in analogy to experiments in animals

*Since writing this paper L. W. Parr and H. Krischner reported a case of a fatality from a transfusion in which both the donor and recipient belonged to Group O (Hemolytic Transfusion Fatality With Donor and Recipient in the Same Blood Group J. A. M. A. 98:47, 1932).

In fact some cases were reported in which blood clinically compatible at the first transfusion caused disturbances when used repeatedly. This phase as well as the findings of abnormal isoagglutinins after several transfusions requires further investigation. There is little doubt that transfusion could incite the production of isoantibodies when incompatible blood is injected which is tolerated at first by virtue of low agglutinin content of the recipient's serum." This appears to be substantiated by one of the cases reported by Astiowe. In this case the recipient (a child eighteen months of age) belonged to Group O, but received blood from its mother who belonged to Group A. The first transfusion was well tolerated because the isoagglutinin had not yet become active. A subsequent transfusion from the same donor, however, resulted in an almost fatal hemolytic reaction accompanied by severe shock. Apparently the first transfusion incited the production of isoantibodies which were responsible for the reaction, or it might have been that these isoantibodies became active in the interim between the first and second transfusion. Previously, Lindeman⁴⁹ also cited a case in which an hemolytic reaction occurred five days after a second transfusion of blood from the same person who acted as donor in the first transfusion, which was uneventful. Subsequent tests showed hemolysis of donor's cells by the patient's serum. More recently, Landsteiner, Levine and Janes⁵⁰ reported a case in which an abnormal isoagglutinin was found in the serum of a patient after the first and second transfusion and which reacted on numerous blood specimens, including cells of Group O, and those of the donor. In this case, however, there were no posttransfusion reactions. Hemolytic reactions even after the first transfusion of compatible blood have also been observed. Carrington⁵¹ cites a case in which the recipient developed hemolysis soon after a transfusion of blood from a donor who had been previously tested and found to be compatible. It is quite certain that such instances, although not frequently reported in the literature, are not of infrequent occurrence.

UNCLEAN APPARATUS

Unclean apparatus is known by all who perform blood transfusions to be frequently the cause of reactions. This may be due to the powder of new rubber tubing, accumulated blood clots in the apparatus employed (especially if the latter is a complicated one), debris which remains in the apparatus or needles after sterilization, etc. The tendency recently has been to simplify the apparatus with the aim of eliminating the possibilities of such collections of extraneous matter." " Passing saline through the apparatus just before the transfusion will prevent accidents of this type.

CITRATE

Since the demonstration by Agote⁵⁴ in 1914, and the independent observations by Weil⁵⁵ and Lewisohn⁵⁶ in 1915, that sodium citrate may be used as an anticoagulant in blood transfusions, there has arisen a great controversy regarding the advantages and disadvantages of the use of citrate intravenously. Much has been written pro and con. In 1917, Sydenstricker⁵⁷ found only 17 per cent posttransfusion reactions when citrate was used. Ravdin and Glenn⁵⁸

from a study of 161 citrate transfusions, concluded that the simplicity of this method warranted its preference over others in vogue at that time. They encountered as many chills with the use of paraffin glass cylinders as with sodium citrate. In a similar review of 269 cases studied by Lewisohn⁵⁹ the latter concluded that citrate transfusions were as satisfactory as any other method employed at that time, which at best were crude as compared to the perfected methods of today. As regards the production of hemolysis by citrate, O'Malley and Hartman,⁶⁰ in the light of their work on citrated plasma in the treatment of pneumonia, concluded that the hemolysis is due not to the sodium citrate but to contact of the blood with the wall of the container, resulting in trauma to the platelets with subsequent thrombin formation. In a subsequent publication, Hartman⁶¹ reiterated this point and cited the experiments of Minot⁶² and the observations of De Kruif⁶³ to substantiate this opinion.

The general toxic effect of citrate in the blood stream is also a controversial question. Henderson,⁶⁴ in a letter to Joannides⁶⁵ who, in 1924, was investigating this problem, agreed with the latter that the use of citrate in such small quantities as are employed in blood transfusions is not dangerous. Giehner⁶⁶ found that citrate is not toxic when used in even larger doses. Rous and Turner⁶⁷ furthermore, were of the opinion that not only is citrated blood harmless but even of particular advantage during war time when it becomes necessary to have a constant supply on hand since it is possible to preserve the blood for a month if prepared in the form of a mixture of dextrose and sodium citrate (3 parts of blood, 2 parts of 3.8 per cent sodium citrate and 5 parts of 5.4 per cent dextrose in water). Hoffman⁶⁸ also considered citrate transfusion a useful procedure and believes that the deleterious effects and even the fatalities which have been ascribed to citrate are not due to citrate per se, but to other causes, such as poor technique, bad risks, etc.

There are, however, many observers who decry the use of citrated blood. Thus, Brines⁶⁹ states that there is never a time when citrate is as good as whole blood. Bacon⁷⁰ deprecated the use of citrated blood, asserting that it carries with it "heavy liabilities." Beinheim⁷¹ has been even more emphatic in his warning, an abstract of which follows: "It was thought that increased familiarity with this method will decrease the evils. The dread reactions still persist, and persist despite the most painstaking efforts to discover their cause or origin, despite the most careful mixing of citrate with blood, despite the most carefully planned and executed citrate transfusions carried out by men whose long experience with general blood transfusions would preclude the possibility of technical error." After citing several fatalities, as a warning to others, he continues, "So I maintain that this reaction following the citrate transfusion with its inherent danger and failure of medical men to take cognizance of its importance, is analogous to the story of our erstwhile indifference to blood tests, and I am trying to sound a warning." It has also been charged by Unger^{72, 73} and others that citrate destroys platelets, that it develops anti-complementary properties in plasma, that it reduces the phagocytic and opsonic index of the blood, that the red blood corpuscles are made more friable, and that it produces a general systemic reaction with malaise and chills. That such reactions are common is apparently borne out by the observations of numerous

investigators Kietzler⁷⁴ found that reactions are more frequent when citrated blood is used than when even so crude a method of unmodified blood transfusion as that of Lindeman is employed. Pauchet⁷⁶ encountered disagreeable chills with citrated blood and was forced to resort to a method in which paraffin coated syringes were used instead of citrate. Platt,⁷⁶ Moons⁷⁷ and Heilmann⁷⁸ have each described blood transfusion fatalities which they believed to be due to the use of citrate. Beinheim⁷⁹ found that despite precautions, reactions occurred in 20 to 40 per cent with refined methods of whole blood transfusion. Meleney et al⁸⁰ encountered reactions in over 64 per cent of 196 cases in which citrate transfusions were performed. Lederer,⁸¹ in a comparative study of a consecutive series of 100 transfusions of citrated and whole blood transfusions, encountered about 50 per cent reactions with citrated and none with whole blood transfusions. More recently, a similar study conducted by Landon⁸² revealed that reactions occurred five times as frequently with citrated as with whole blood.

The exact causes for these reactions are still unknown. According to McClure,⁸³ they are due to the prolonged exposure of the blood to foreign substances that the citrate method necessitates. The experimental investigations and extensive practical experience of Dinker and Buttingham⁸⁴ have led them to conclude that the reactions are due to the deleterious effects of the citrate on the blood platelets. Horsely et al⁸⁵ are of the opinion that citrate produces certain chemical and biologic changes in the blood, which changes may account for the reactions. Whichever is the correct explanation, it is quite obvious from the review of the literature, that reactions are much more common with citrated than with unmodified blood transfusions.

INCIPIENT COAGULATIVE CHANGES

Reactions, however, occur with none of the aforementioned causes to explain them. Some of these resemble the reactions which are due to hemolysis or agglutination, yet present no evidence of the existence of either of these phenomena. Such reactions have been ascribed to the incipient coagulative changes in the transfused blood before it enters the circulation. From a study of the effects of physical influences on blood, Satterlee and Hooker⁸⁶ have been led to conclude that such altered blood contains potential coagulative factors, such as thromboplastin and thrombin, which probably produce coagulation after reaching the circulation, yet the changes are too fine to be detected by the ordinary methods. Similar views are held by Clough,⁸⁷ Horsely⁸⁸ and others. For want of a better explanation the above must be accepted as accounting for this type of transfusion reaction. The experienced transfusionist who has seen the frequently unavoidable agitation, whipping and other abuses to which the blood is exposed before it is injected into the recipient's vein, can best appreciate the logic in such an explanation.

ALLERGIC REACTIONS

Allergic reactions to blood transfusions are not uncommon. Carington⁸⁹ reports a case in which the patient, within ten hours after receiving 50 cc of compatible citrated blood, developed dyspnea, laryngeal stridor, cyanosis, pul-

monary edema, became comatose, and died. There was no hematuria or hemoglobinuria. Blottner⁹⁰ cites several instances of shock from repeated transfusions of compatible blood. The nature of these reactions, the presence of eosinophilia, and the fact that the reactions in these cases occurred with those transfusions which were performed in three to six weeks after the first, indicated that they were allergic in nature. In reviewing the results of 700 transfusions, Duke⁹¹ states that allergy is an important contraindication. The allergic phenomena, according to Hanzlik,⁹² depend upon a disturbance of the physical and chemical (colloidal) mechanism of the blood and tissue. According to Kordenat⁹³ most of the so-called "anaphylactic reactions" are really due to protein shock, which may occur in one of two ways. First, the donor's blood may increase the protective or cleavage power of the patient's serum so that there is cleavage of damaged tissue which produces the reaction. Secondly, because of poor proteolytic ability of the liver, partly altered proteins leak through, resulting in parenteral cleavage and nonspecific protein shock (Widal crisis). Anaphylactic reactions have occasionally been ascribed to food allergy. Thus, Duke and Storer⁹⁴ encountered reactions in sensitive persons who received blood from donors who ingested the offending food before the transfusion. This theory has been partially substantiated in a report by Biern⁹⁵ who claims that such reactions were prevented by using fasting donor's blood. Hustin⁹⁶ believed that anaphylactic reactions to foreign protein can be warded off by preliminary injections as described by Besiedka. In our experience, adrenalin has been found extremely helpful in warding off and combating most of the so-called allergic reactions.

In concluding the very brief review on this phase of the subject, it may be stated that the information thus far gathered is very unsatisfactory. According to the most recent opinions held by the foremost investigators of hypersensitiveness, it is questionable whether the reactions described above are truly allergic. Urticaria, eosinophilia, the effect of adrenalin in controlling the symptoms are not necessarily signs of an allergic phenomenon. It is not within the scope of this paper to delve into the intricacies of the subject. For a comprehensive discussion of this phenomenon the reader is referred to the works of Coca and to those of Welch and Karsner.⁹⁷

SYSTEMIC DISEASES

The transfusion of blood may have deleterious effects on the kidney with resultant severe and frequently fatal reactions. An excellent review of this subject was recently written by Boardley⁹⁸ who described 3 cases of this type, 2 of which were fatal, and also cited about 22 other cases previously reported by other authors. In sixteen of these the reactions were fatal. According to Brines, the reactions occurring in these persons were due to underlying renal disease. Thus Schumacher⁹⁹ reported 2 cases in which blood transfusion precipitated fatal uremia in patients who were suffering from previous renal impairment. A greater number of the cases previously mentioned, however, showed absolutely no evidence of renal impairment before the transfusion. We have also found the converse to be true. Many patients with nephritis some

of them with marked retention, have received transfusions without any untoward results

A review of the literature on this point confirms this observation. Ottenberg¹⁰⁰ carried out a large number of transfusions on patients with diseased kidneys, and several with one kidney, with no untoward results in any. Mosenthal¹⁰¹ failed to notice a suppression of urine after transfusions on patients with Bright's disease. He believed that anaphylaxis and hemoglobinemia from incompatible blood cause the damage to the kidney, but that the effect is the same in the normal person as in those with Bright's disease. On the contrary, favorable results, following transfusion in cases of renal disease, have been reported by Ramsay,¹⁰² Martin,¹⁰³ Iversen,¹⁰⁴ and Flandin.¹⁰⁵ In nephrosis likewise, no contraindication to transfusion has been reported. Epstein,¹⁰⁶ Clausen,¹⁰⁷ and others failed to find any unfavorable results in these cases, and in fact recommend it as a valuable therapeutic measure.

The cause of the renal involvement is still not understood, and is an extremely interesting problem. Boardley believes that it is all due to incompatible blood, although definite incompatibility was established in only 6 of the 17 cases which he describes in more or less detail. One of his own fatal cases, furthermore, received blood which was found by previous cross agglutination tests to be compatible with the recipient's blood. One of us (P) also had an experience with a fatal case in a patient of this type who received blood which, as far as could be determined by the usual methods of cross agglutination, was compatible with that of the recipient. To ascribe all reactions of this type to incompatible blood would, therefore, not account for these rare instances, and a more satisfactory explanation is still wanting. The anatomic changes in these cases have as yet offered little information in that respect. The outstanding changes in the kidneys consist of swelling and advanced degeneration of the tubular epithelium (many of which contain a peculiar brownish pigmented material which is believed by some to be of hemoglobin origin). Masses of blood pigment, as well as debris, leucocytes, wandering cells, and desquamated cells are found in the lumina of the tubules. Dilatation of the glomerular capsules and an interstitial cellular infiltration occur in some of these cases. In the liver, central necrosis is the common finding.

The presence of the masses of hemoglobin pigment in the renal tubules is the basis for the theory that the blockage of the tubules by these casts produces a renal insufficiency. But as has been definitely shown by Scollards and Minot¹⁰⁸ and later by Rich,¹⁰⁹ the injection of hemoglobin into human beings failed to produce renal impairment. At this point, it may be interesting to cite the experimental evidence of Baker. He showed that in these cases hemoglobin is thrown out in solution when the P_{H} of the medium is 6 or less and when the sodium chloride content is 1 per cent or over. Under such circumstances, he claims, hemoglobin is excreted in solution in the glomerular transudate and after concentration in the tubules the pigment is precipitated in the form of hematin, due to the acidity and increase in salt concentration. According to Baker, this condition may, in fact, be combated by producing alkaline diuresis.

The changes noted in the tubules of the kidney, which are so similar to those of the so-called tubular nephritis, described by Brown et al.¹¹⁰ as occurring in

pyloric obstruction with vomiting, has lent support to the theory that the post-transfusion renal insufficiency follows a loss of chlorides incident to vomiting and its consequential nitrogen retention.

Acute poisoning by an irritating or toxic substance as being a cause of post-transfusion renal insufficiency is suggested by the evidences of inflammatory reaction observed in the kidney (leucocytic infiltration, edema, necrotic tubular epithelium), and also by the foci of necrosis in the liver.

From a clinical point of view, the nature of the symptoms of the reaction favors some and negates others of the theories mentioned to explain the causative factors. Thus, the theory of toxic irritation is strengthened by the similarity between the symptom complex of black water fever and that of the transfusion reaction, while the theory of hemoglobin blockage of the tubules is weakened by the absence of hemoglobinuria in some of these reactions. The similarity between the reactions to transfusions and shock which is frequently associated with severe renal insufficiency, as pointed out by Lougeope and Rackemann¹¹¹ has led to the consideration of a theory which ascribes the reaction to shock resulting from a kidney sensitiveness to incompatible blood. This phase has already been considered in the discussion of allergic reactions.

Other organs besides the kidneys may be overtaxed by blood transfusions. As Wildegans¹¹² recently emphasized, the heart, liver, and blood vessels may all suffer serious injury. Bisenberger¹¹³ reported a case of sudden cardiac paralysis after the injection of only 150 c.c. of blood. This was a case of mitral stenosis in which the injury to the heart was proved at autopsy. Probably numerous other fatalities have occurred due to overtaxing of the heart especially in those persons whose cardiac reserve is at a low level. In this connection, Eyster's¹¹⁴ experiments are of interest. From an x-ray study of the silhouette area of the heart he found that in the recipient, increments of blood by transfusion in amounts to within 1 per cent of body weight resulted in a transitory alteration of the cardiac size. Although it was found that the compensatory mechanism in the cases studied, caused a rapid adjustment to the normal circulatory conditions, notwithstanding the altered blood volume, it is questionable whether a diseased heart would show a similar compensatory ability. We have frequently observed a severe type of reaction which is most probably due to overdistention of the right side of the heart, with what appears to be an interference to the proper return flow of the cerebral circulation. That the reaction is cardiac in nature, is evidenced by the fact that the patient suddenly becomes pulseless and cyanotic, and the cardiac sounds become almost inaudible. At the same time there is a loss of consciousness, rolling of eyeballs, and in some instances muscle spasms. This is soon followed by cold and clammy perspiration and then simultaneously with the cessation of the transfusion, a gradual return to consciousness. The patient "wakes up" from a brief "sleep." All this transpires in a period of a minute or two, provided the symptoms are recognized early enough and the transfusion is halted. Failure to observe the onset of this reaction quickly enough may prove fatal to the patient, or result in a prolonged reaction, so that unconsciousness may persist for as long as ten or fifteen minutes, and a much slower return of the heart action to normal. This type of reaction occurs in persons with poor myocardial tone. In performing trans-

fusions on such patients, therefore, it is extremely important, to watch the pulse and to inject the blood slowly and guardedly

TRANSMISSION OF DISEASE

The transmission of disease from donor to recipient and vice versa, may be considered a reaction to blood transfusion. This hazard has recently been pointed out by a number of observers. Transmission of asthma has been reported by Ramirez¹¹, measles, by Baugess¹¹⁶ and Harrell¹¹⁷, smallpox, by Blacklock¹¹⁸, malaria, by Korabelnikoff¹¹⁹, Flaum¹²⁰ and others, and syphilis, by a number of observers including ourselves.^{121 122} It may be added that syphilis has similarly been transmitted to the donor. In order to avoid these complicating reactions, rigid physical and serologic examination of family as well as professional donors has been urged.

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SPECIAL FEATURES OF THE BLOOD IN INFANCY AND CHILDHOOD*

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IN INFANCY and childhood the hemopoietic system undergoes changes which lead to the adult condition. The bone marrow produces red and white blood cells side by side, and fills all of the bones. It is only with onset of adolescence that the red bone marrow begins to recede from the shafts of the long bones and be replaced by fat. Just as the red bone marrow may replace this fat during severe anemias in adults and thus revert to the childhood type, so the bone marrow of infants more easily reverts to the fetal type under the stress and strain of hemopoietic stimuli or irritants. This apparent difference in response to similar disease processes has given rise to the impression that blood dyscrasias of children are quite different from those of adults and represent separate disease entities. There are additional features of the normal infant's bone marrow and blood, an appreciation of which is important in evaluating the different blood pictures.

Anatomy of the Bone Marrow—The anatomy of bone marrow varies considerably at different age periods. Piney¹ pointed out that at birth and for the first three to four years, the cut section of the marrow is pink, and the expressed tissue a rich red. At the age of seven years the marrow is less pink and the surface appears greasy. The expressed tissue has distinct fat droplets, however, no macroscopic areas of fat are visible until the age period of twelve to fourteen years. At this time fatty patches occur at the middle of the shafts of the long bones. Within twelve months other smaller fatty areas are noted throughout the marrow. These same changes occur in the tibia, fibula, femur, radius, ulna, and humerus. The fatty changes occur much more rapidly in the lower extremities and, further, these changes are more rapid in the distal portions of the bone than in the proximal. When the tibia and fibula are completely filled with fat, the upper end of the femur still retains red marrow. This is the adult picture as it exists in the long bones. The epiphyses also become entirely fatty by a similar process as previously described. Red marrow is always to be found in the ribs, sternum, vertebrae, os innominatum, and in the bones of the skull.

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The adult marrow has a volume of about 1400 cc, which is far in excess of the actual amount required for maintaining a normal physiologic function. As a result, a large reserve space is present which is potentially available for the formation of blood cells under abnormal conditions. The marrow of the infant lacks this reserve. For the body of the infant to compensate for an anemia it has two mechanisms: 1, the displacement of fluids, blood and plasma, 2, the resorption of bone. These two processes safeguard the function of the marrow under normal conditions and to a certain extent under pathologic conditions. However, with increasing abnormal demands which the adult could care for by his "reserve," the infant is unable to compensate for and the marrow suffers as a result. The added load is too great, and the immature cells of the marrow are thrown out into the stream long before they are ripe, giving the typical embryonic type of peripheral blood. In severe anemias, foci of blood formation may reappear in the liver and spleen. The blood picture of anemia in the adult after a prolonged period of time may simulate that of the infant, but the immediate pictures differ, since the adult has the mechanism of "reserve."

Hemoglobin in Infancy and Childhood—The majority of the reports in the literature concerning the hemoglobin estimations of infancy and childhood are usually given in "per cent" with no data as to the value of 100 per cent in absolute figures. Some authors have given average figures for the various age groups, omitting the number of cases studied. In Table I are listed the average

TABLE I

AGE	AMOUNT OF HEMOGLOBIN GRAMS PER 100 C C	PERCENTAGE OF HEMOGLOBIN (14 GRAMS = 100 PER CENT)	NUMBER OF CASES
1 Day	19.23	137%	103
2 Days	16.23	116	40
2-3 Days	22.05	158	45
3 Days	15.32	113	30
4 Days	16.19	116	39
4-8 Days	20.99	149	47
5 Days	15.90	113	34
6 Days	15.43	110	35
7 Days	15.29	109	29
8 Days	14.26	101	34
9 Days	14.30	101	29
10 Days	12.50	89	26
11 Days	13.83	99	31
12 Days	12.74	91	12
13 Days	17.40	112	1
9-13 Days	20.89	149	38
24 Days	14.40	103	1
14-56 Days	17.00	121	46
3-5 Months	13.30	95	42
6-11 Months	13.30	95	43
12 Months	12.57	89	34
11-23 Months	11.90	85	12
2 Years	12.57	90	33
3 Years	13.16	94	31
4 Years	13.62	97	31
5 Years	13.54	97	35
12 Years	11.06	79	7
13 Years	11.20	80	33
14 Years	11.48	82	17
15 Years	12.32	88	27
16 Years	12.32	88	13

hemoglobin content in grams per 100 c.c. and in equivalent percentages (using 14 grams as 100 per cent) of 777 healthy children collected from the literature and 100 cases observed by the author. The periods covered are from birth to the fifth year, and from the twelfth to the sixteenth year.

For the first ten days the hemoglobin ranges from 22.05 grams to 12.5 grams per 100 c.c. of blood with the average well above that found in adults. Gradually it falls to a level of 11.9 grams, being the lowest from the third month to the second year. There is then a slow return to the adult range with a slight drop again at puberty.

Red Blood Cells—The number of red blood cells present in the peripheral blood seems to parallel the amount of hemoglobin. At birth, and for the first few weeks following, the count may be as high as seven million red blood cells per cubic millimeter, the average being about five and a half million per cubic millimeter. From the first month to the sixteenth year, the average count is approximately four and a half million per cubic millimeter. The results com-

TABLE II

AGE	RED BLOOD CELLS MILLIONS PER CU. MM.	NUMBER OF CASES	
Birth	6.83	87	(Umbilical cord cut immediately— 6 cases 5.08 Umbilical cord cut after arterial pulsations cease— 8 cases 5.57) Hayem
$\frac{1}{2}$ Hour	5.19	71	
6 Hours	5.66	71	
11 Hours	4.57	1	
12 Hours	5.52	71	
18 Hours	5.38	71	
24 Hours	5.65	155	
30 Hours	5.37	71	
36 Hours	5.43	71	
48 Hours	5.43	110	
3 Days	5.29	30	
13 Days	4.83	30	
4 Days	5.43	38	
5 Days	5.21	31	
6 Days	5.14	34	
7 Days	5.15	29	
8 Days	4.73	33	
9 Days	5.36	36	
10 Days	4.65	26	
4-10 Days	4.65	30	
11 Days	4.73	31	
12 Days	4.50	12	
13 Days	3.69	1	
14 Days	5.79	10	
24 Days	3.40	1	
3 Months	4.50	17	
1 Year	4.00	10	
2 Years	4.30	10	
5-10 Years	5.00	10	
12 Years	4.66	7	
13 Years	4.68	33	
14 Years	4.77	17	
15 Years	4.75	27	
16 Years	4.66	13	

piled in Table II have been collected from data on 1,198 healthy children obtained from the literature and 100 cases investigated by the author.

At birth and the following few days, nucleated red cells are present in decreasing numbers. Neumann² believed that they are common in the newborn. Fischl³ found them present in one case, and absent in two. Hayem and Luzet⁴ claim that erythroblasts disappear from the blood of the fetus during the later weeks of pregnancy, while Hock and Schlesinger⁵ found them present in healthy children. Most authors believe that nucleated red cells disappear by the fourth day. At birth the reticulocytes vary from 5 to 10 per cent (Friedlander and Wiedemann,⁶ Seydewitz and Juergens¹¹). Anisocytosis, poikilocytosis, and polychromatophilia are also marked in the blood of the newborn. The various signs of regeneration assume normal proportions within a few days. In addition, some authors have reported the presence of the so called "ghost cells" at birth. Their significance is not known.

White Blood Cells—In the newborn, the number of white blood cells present in the peripheral blood is markedly elevated. It may total as high as forty thousand per cubic millimeter, the average being twenty thousand per cubic millimeter. Up to the eighteenth hour, there is a slight increase in the total number of white cells, followed by a gradual decrease. (This high white cell count probably includes the nucleated red blood cells which are present.) All during infancy and early childhood a slight leucocytosis is present, the average number of cells being 12,000 to 14,000 per cubic millimeter. About the tenth year the normal, adult count is reached. In Table III are listed the averages of the various age groups.

TABLE III

AGE	TOTAL WHITE BLOOD CELLS PER CU MM	NUMBER OF CASES
Birth	18,100	58
$\frac{1}{2}$ Hour	16,600	71
6 Hours	21,000	71
12 Hours	22,500	71
18 Hours	21,200	71
24 Hours	17,700	130
30 Hours	17,600	71
36 Hours	15,400	71
48 Hours	12,200	143
72 Hours	12,900	57
96 Hours	10,100	76
5 Days	9,120	61
6 Days	11,200	61
7 Days	11,500	38
8 Days	11,700	58
9 Days	12,100	57
10 Days	12,400	45
30 Days	12,200	18
2 Months	13,500	3
3 Months	13,200	2
4 Months	11,200	12
5 Months	13,200	2
6 Months	13,000	2
1 2 Years	6,500	150
2 3 Years	7,350	150
3 4 Years	6,400	150
4 5 Years	6,560	150

There have been many theories presented in explanation of the high leucocyte count. Some authors believe it to be due to the loss of body fluids, others as due to trauma during delivery, while still others believe it the result of bone marrow stimulation, or a reaction from ingested protein after eating for the first time.

Platelets—Of all the cellular elements derived from the bone marrow in the peripheral blood, the platelets are the only ones not increased in number. They average from two hundred to four hundred thousand, and this amount remains practically constant throughout life.

In addition to the theories presented in explanation of the leucocytosis, several others have been offered to account for the increased numbers of red cells, hemoglobin percentage, and white cells of the newborn. Lepine⁷ believed the variation due to changes in the plasma volume, Hayem⁸ accounted for the increased numbers as the result of a new formation of the elements. In addition, he demonstrated a variation in the number of red blood cells when the umbilical cord was cut immediately and after waiting for the arterial pulsations to cease. In fourteen observations there were approximately one-half a million red cells per cubic millimeter more in samples of blood obtained after the pulsations ceased. Schiff⁹ believed the blood to be concentrated as a result of the loss of body fluids. Elder and Hutchinson¹⁰ are inclined to agree with Hayem, for the figures observed varied too much within a few hours to be accounted for by either increased cell production or hemolysis. If the bone marrow were stimulated as the result of lack of oxygen or increased body need, or if the blood were concentrated from the loss of fluid, the platelets should also be increased in amount.

The presence of immature red blood cells and white blood cells in the peripheral circulation for only a few days, in addition to the fact that the platelets remain constant, leads one to account for the newborn blood picture by some other method. This has been suggested by Schilling,¹¹ and it is thought to be clearing of the liver and spleen as foci of hematogenesis.

Differential (Leucocyte Count)—During the first twenty-four to forty-eight hours, the polymorphonuclear leucocytes are present in abundance, assuming the proportion seen in adult life. After two or three days, they decrease from a total of about 60 per cent to 35 or 40 per cent. The eosinophils average 3 to 7 per cent, while the basophils remain about 0.5 per cent. The mononuclears average 3 to 10 per cent. The difference is made up with small and large lymphocytes. The types of cells present after the first few days are all adult in nature. This factor assumes great clinical value when estimating the importance of the blood as a means of diagnosis. Table IV is a summary of the average differential counts of many observations previously reported.

Smith¹² has investigated the presence of lymphocytosis in 37 infants, 21 of whom were less than one year old. He made comparative differential counts in living preparations and stained smears. The percentage of polymorphonuclear leucocytes was 8.6 per cent more in the fresh preparation than in the fixed film, and further, the number of lymphocytes in a dry smear was 11 to 14 per cent more than those in a living sample. This difference he believes is due to the failure of the observer to identify properly the mashed cells present in the coverslip preparations and the unequal distribution on the Wright

TABLE IV

AGE	POLY MORPHONUCLEAR NEUTROPHILS	EOSINOPHILS	BASOPHILS	LYMPHOCYTES	MONOCYTES
	%	%	%	%	%
1 Hour	50.4	2.2	0.2	44.5	2.3
6 Hours	59.1	1.4	0.2	36.4	2.7
12 Hours	66.0	1.2	0.2	30.1	2.6
18 Hours	63.6	2.0	0.1	32.0	2.0
24 Hours	60.6	2.6	0.2	29.5	4.2
36 Hours	51.8	3.0	0.1	42.8	2.2
48 Hours	54.9	2.1	0.2	35.0	5.3
72 Hours	49.0	2.4	0.2	39.0	7.5
96 Hours	43.5	2.4	0.2	45.0	8.1
5 Days	41.0	3.0	0.2	46.0	9.0
6 Days	37.0	3.8	0.1	50.0	9.5
7 Days	35.0	4.5	0.15	52.5	9.5
8 Days	24.5	2.8	0.15	63.0	10.0
9 Days	31.5	4.3	0.0	55.5	10.5
10 Days	26.0	1.9	0.25	63.0	9.2
11 Days	26.0	1.7	0.25	61.5	10.5
12 Days	30.0	2.0	0.5	62.0	5.5
2 1/4 Days	32.9	6.2	0.2	52.0	10.1
2 1/2 Weeks	29.7	4.9	0.4	56.3	9.0
1 1/2 Months	29.8	6.1	0.46	53.8	12.0
1 1/6 Months	34.5	3.5		50.0	11.0
2 1/6 Months	26.3	2.6	0.30	60.0	10.0
6 1/2 Months	35.0	1.5	0.3	52.0	9.5
1 1/2 Years	41.9	3.0		47.0	4.0
2 1/3 Years	48.2	3.9		38.4	4.5
3 1/4 Years	52.6	5.7		33.2	4.2
4 1/5 Years	61.0	6.3		25.8	3.7

stained films. With the above correction, the differential count seen in infancy and childhood would more closely approximate that of the normal adult. This would mean, however, that polymorphonuclear neutrophils in babies were more fragile than those of the adult and that the physical properties of their blood were also different.

Discussion—A review of the various blood pictures described indicates a slow transition from the blood picture of infancy and childhood to the normal picture of the adult. However, there are several features of the bone marrow and blood of infancy and childhood which are outstanding: 1, the distribution of the bone marrow; 2, the changes in hemoglobin values; 3, a polycythemia at birth and for about one month following; 4, a persistent leucocytosis; 5, the presence of immature red and white blood cells in the peripheral blood during the first few days of life; 6, lymphocytosis up to the second or third year.

In addition to the above-mentioned features is the abnormal response of the bone marrow in infancy on the slightest provocation with a reversion to the embryonic type of blood. Infection in adults is manifested by a leucocytosis, whereas in infants, not only is a leucocytosis present, but many immature white cells are thrown into the stream with a resulting leucemoid picture. Anemias in adults, unless severe, rarely if ever show immature red cells in the peripheral blood, because of the "reserve" of the bone marrow (the replacement of fat marrow by red marrow). In infancy and childhood there is no "reserve" of the bone marrow, and any extra demand results in an overactivity which turns out only increasing numbers of immature red cells to the blood. Normoblasts,

megaloblasts, anisocytosis, poikilocytosis, and polychromatophilia are much in evidence. The liver and spleen often assume the rôle of hemopoiesis which they formerly had in the fetus. The understanding of this vague mechanism is most important in evaluating the confusing blood pictures occurring in infancy and childhood.

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DIAGNOSTIC FEATURES OF THE BLOOD COUNT AND OF THE MORPHOLOGY OF THE BLOOD IN DISEASES ASSOCIATED WITH SPLENOMEGALY*

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PHYSICIANS intimately concerned with clinical hematology have long recognized minor differences in blood counts which they have more or less intuitively used as aids to diagnosis, however these minor characteristics have rarely been analyzed. Likewise, hematologists interested chiefly in examination of the blood have described many variations from the normal, which however, have not been sufficiently simplified to come within the grasp of the practicing physician. I hope that this paper may at least partially clarify this situation. Of the diseases associated with splenomegaly, the commonest only will be considered.

HEMOLYTIC ICTERUS

An examination of blood, made February 10, 1931, gave the following results:

Hemoglobin	8.5 gm. in 100 c.c. (51 per cent)
Erythrocytes	2,300,000 in 1 c.mm. of blood
Color index	1.1
Leucocytes	7,400 in 1 c.mm. of blood
Lymphocytes	27 per cent
Monocytes	3 per cent
Neutrophils	66.5 per cent
Eosinophiles	2.0 per cent
Myelocytes	1.5 per cent
Normoblasts	5 seen
Reticulated erythrocytes	13.4 per cent
Platelets	134,000 in 1 c.mm. of blood

Comment on blood picture: Moderate to marked microcytosis, cells spherical, evidence of active regeneration.

Marked anemia was present. The color index of 1.1 was higher than that seen in simple secondary anemia, and not so high as that which usually occurs in cases of pernicious anemia. Not infrequently the color index in cases of hemolytic icterus is 0.8 or 0.9. Occasionally, in very severe cases, with extreme anemia, it is greater than 1.0. If repeatedly the color index is 0.8 or 0.9, hemolytic icterus may be suspected from this feature alone.

The number of leucocytes was slightly above normal, whereas the percentage of polymorphonuclear neutrophils was normal. These features are suggestive of active regeneration of the bone marrow, as is also the presence of an occasional neutrophilic myelocyte.

The percentage of reticulated erythrocytes was very much above normal. This is the most trustworthy sign of active production of erythrocytes. The

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reticulated cells are regarded as young erythrocytes not yet completely saturated with hemoglobin. The reticulation is due to a reaction between a vital stain (brilliant cresyl blue) and the basophilic spongioplasm which has not entirely been replaced by hemoglobin.

The combination, therefore, of a moderately high color index with a normal leucocyte count or slight leucocytosis, a normal or increased percentage of polymorphonuclear cells, and a very high percentage of reticulated erythrocytes, is almost pathognomonic of hemolytic icterus, which is a disease of increased hemolysis accompanied by very active regeneration of blood.

Microcytosis, reported after examination of the blood smears, is a most valuable characteristic from the diagnostic standpoint. A very high percentage of microcytes, 60 or 70 per cent, is almost always seen in cases of hemolytic icterus. The microcytes are usually spherical, and appear as deeply stained cells well filled with hemoglobin. In addition to microcytes, large, oval macrocytes are also seen, their presence however, is not essential to the diagnosis. The blood smears in cases of hemolytic icterus also show signs of very active regeneration of erythrocytes, especially polychromatophilia and occasional normoblasts. There is a shift to the left of polymorphonuclear leucocytes, that is, a decrease in the number of lobes of the nuclei, possibly 30 per cent or more of the polymorphonuclears may have only two lobes. There may also be evidence of slight immaturity in the granular series indicating hyperactivity of the bone marrow.

SPLENIC ANEMIA

An examination of blood, made March 19, 1930, gave the following results

Hemoglobin	5.5 gm. in 100 c.c. (33 per cent)
Erythrocytes	2,225,000 in 1 c.mm. of blood
Color index	0.7+
Leucocytes	2,300 in 1 c.mm. of blood
Lymphocytes	46.5 per cent
Monocytes	1.5 per cent
Neutrophiles	52 per cent
Platelets	124,000 in 1 c.mm. of blood
Reticulated erythrocytes	0.5 per cent

Comment on blood picture. Marked poikilocytosis with hypochromasia, and apparent slight reduction in platelets.

A diagnosis of splenic anemia is made chiefly by exclusion of other diseases associated with splenomegaly. The features of the blood count are only slightly suggestive and by no means constant. Those features which are not infrequently of diagnostic value are exemplified in the blood count which has been presented. There is marked anemia, with a color index of 0.7+. There is definite leucopenia and slight increase in the percentage of lymphocytes, associated with slight decrease of the percentage of neutrophiles. That these alterations in the leucocyte and differential counts do not mean a hypoplastic condition of the bone marrow is evidenced by presence in the blood smear of reticulated erythrocytes and absence of features of inactive regeneration.

In blood smears, on morphologic examination, there not infrequently is marked poikilocytosis, without, however, the marked macrocytosis which is seen

in pernicious anemia, moreover, erythrocytes reveal hypochromasia. The marked poikilocytosis may be regarded as evidence of toxicity, and possibly of abnormal destruction of blood in splenic anemia. Although poikilocytosis is marked only in cases of splenic anemia with severe anemia, it is a more prominent feature than in secondary anemia from hemorrhage.

The diagnosis of splenic anemia sometimes is made in cases in which the leucocyte count is normal or there is even slight leucocytosis. Potential splenic anemia may not be accompanied by reduction in hemoglobin and number of erythrocytes. However, the features which have been considered are sufficiently constant to assist in the diagnosis.

HEMORRHAGIC PURPURA

An examination of blood, made March 24, 1928, gave the following results:

Hemoglobin	27 per cent (Dire)
Erythrocytes	2,060,000 in 1 c mm of blood
Color index	0.7-
Leucocytes	1,800 in 1 c mm of blood
Lymphocytes	33 per cent
Transitionals	2 per cent
Neutrophils	62 per cent
Eosinophiles	2 per cent
Basophiles	1 per cent
Reticulated erythrocytes	9.3 per cent
Platelets	50,000 in 1 c mm of blood
Bleeding time	30 minutes
Clot retraction	Slight after six hours

Comment on blood picture: Definite evidence of active regeneration, with anisocytosis, polychromatophilia, and many reticulated cells, platelets much reduced in number.

Marked anemia of the secondary type was present. The leucocyte count was slightly below normal, which might in itself suggest reduced activity on the part of the bone marrow, however, in conjunction with a normal percentage of neutrophils and a high count for reticulated cells, it would not be so interpreted. It should be emphasized, therefore, that a slightly low leucocyte count in the presence of other evidence of active regeneration on the part of the bone marrow cannot be regarded as indicative of hypoplastic marrow. The leucocyte count usually is normal or more than normal in cases of hemorrhagic purpura.

The platelet count was low, the bleeding time was prolonged and the retraction of the clot was delayed. These abnormal features of coagulation are seen chiefly in hemorrhagic purpura, acute aplastic anemia, and the hemorrhagic phase of leukemia. Leukemia was excluded by absence of immature cells. It may be said in this connection, that not infrequently in my experience immature cells and even stem cells have been mistakenly classified as monocytes and lymphocytes and care must be exercised not to overlook immaturity. Aplastic anemia was excluded by absence of evidence of hypoplastic marrow, that is, by the high percentage of reticulated cells and normal percentage of polymorphonuclear cells. Consequently, after analysis of such a blood count one would strongly suspect a diagnosis of hemorrhagic (thrombocytopenic) purpura in spite of the presence of slight leucopenia.

Examination of blood films in cases of hemorrhagic purpura reveals the characteristics of active physiologic regeneration in contradistinction to aplastic anemia, in other words, the presence of an appreciable number of reticulated erythrocytes, moderate or marked polychromatophilia, and anisocytosis associated with macrocytosis. Moreover, platelets may not be seen in the films, and usually when platelets are not found the count is below 30,000. If platelets are present they are likely to vary greatly in size and to appear deformed in shape, very large platelets are sometimes present.

ACUTE APLASTIC ANEMIA

An examination of blood, made December 27, 1927 resulted as follows

Hemoglobin	30 per cent
Erythrocytes	1,540,000 in 1 c mm of blood
Color index	0.9+
Leucocytes	2,800 in 1 c mm of blood
Lymphocytes	66.5 per cent
Monocytes	7 per cent
Neutrophiles	26.5 per cent
Reticulated erythrocytes	None
Platelets	52,000 in 1 c mm of blood
Coagulation time	10 minutes
(Lee and White method)	
Bleeding time	7 minutes
Clot retraction	Absent in six hours

Comment on blood picture. Morphologic evidence of decreased regeneration with absence of reticulated cells, platelets reduced in number.

This blood count in a case of acute aplastic anemia is presented chiefly because of diagnostic contrast with the preceding count of hemorrhagic purpura. Slight splenomegaly is sometimes present in cases of acute aplastic anemia. In this blood count marked anemia was present; the color index was moderately high. Leucopenia was found, and this was associated with reduction in the percentage of neutrophiles and increase in the percentage of lymphocytes. Leucopenia, with reduction in percentage and in absolute number of neutrophiles, is an indication of reduced activity on the part of the bone marrow. The absence of reticulated cells is also a feature of hypoplastic marrow. The features of coagulation, that is the prolonged bleeding time and the delayed retractility of clot, together with a low platelet count, are the same as those which might be obtained in a case of hemorrhagic purpura. However, in this count, the definite indications of reduced production of erythrocytes, polymorphonuclear leucocytes, and platelets indicate clearly generalized reduction of activity of bone marrow.

Morphologic study of the blood films in this case also gave evidence of aplastic marrow. The scarcity of reticulated erythrocytes, the absence of polychromatophilia, the low absolute and low relative number of polymorphonuclear neutrophiles and the presence of old many lobed polymorphonuclear cells are all characteristics of importance in arriving at the conclusion that regeneration is inactive. In some of the cases a decision with respect to activity of the bone marrow may be very difficult to make and repeated examinations of the films may be necessary before one can determine definitely the degree of regeneration.

INFECTIOUS MONONUCLEOSIS (ACUTE BENIGN LYMPHADENOSIS, BENIGN
LYMPHOCYTOSIS, GLANDULAR FEVER)

An examination of blood, made October 20, 1927, revealed the following

Hemoglobin	61 per cent (Dare)
Erythrocytes	3,840,000 in 1 c mm of blood
Leucocytes	14,000 in 1 c mm of blood
Lymphocytes	70 per cent
Monocytes	15 per cent
Neutrophiles	25.5 per cent
Eosinophiles	0.5 per cent
Basophiles	0.5 per cent

Comment on blood picture Typical of infectious mononucleosis

Clinically this disease may simulate leucemia, and a mistake in diagnosis may lead to great chagrin. Slight enlargement of the spleen is the rule, marked splenomegaly is not seen.

In this blood count it will be noted that slight anemia was present, with a low color index. There was definite leucocytosis and a percentage of lymphocytes of 70. In view of these findings, the diagnosis of chronic lymphatic leucemia would immediately suggest itself. However, the percentage of lymphocytes is not as high as that which is usually seen in lymphatic leucemia, and the diagnosis would have to depend on the findings on morphologic examination of the films.

On morphologic examination of blood films the picture was characteristic. The lymphocytes, as a rule, were larger than normal, some of them of leucocytoid appearance, many of them contained vacuoles both in the nucleus and in the cytoplasm, and the nuclei showed some tendency to lobulation. The lymphocytes showed no definite evidence of immaturity. In films of this kind vacuoles may be mistaken for nucleoli, and careful study of the nuclear structure of the lymphocyte is necessary, gradual transition between parachromatin and chromatin is the essential feature in recognition of a mature lymphocyte. It is true that occasionally a slightly immature lymphocyte may be seen in a case of infectious mononucleosis, and sometimes judgment must be suspended. The monocytes are also increased in number, and usually during the period of recovery, the percentage of monocytes decreases as that of polymorphonuclears increases. The return to a normal blood picture is usually a matter of weeks, but occasionally abnormalities are present for several months.

LYMPHATIC LEUCEMIA WITH NORMAL LEUCOCYTE COUNT

An examination of blood, April 3, 1927, gave the following results

Hemoglobin	31 per cent
Erythrocytes	2,200,000 in 1 c mm of blood
Leucocytes	6,500 in 1 c mm of blood
Mature lymphocytes	57.5 per cent
Immature lymphocytes	10.5 per cent
Monocytes	3 per cent
Neutrophiles	29 per cent

Comment on blood picture Definite immaturity of lymphocytes

The important feature of this examination of blood obviously is the presence of immaturity of the lymphocytes. Some of them were found to be lymphoblasts or stem cells. The nuclear material of immature lymphocytes is relatively sharply differentiated into chromatin and parachromatin. In spite of a normal leucocyte count, which is not uncommon in acute leucemia, a positive diagnosis can be made from the blood. Slight to marked splenomegaly may be present in cases of lymphatic leucemia. It is unnecessary to present a blood count of chronic lymphatic leucemia with leucocytosis or chronic myelogenous leucemia with leucocytosis. The diagnosis in these conditions is readily made. In the acute and subacute forms of leucemia, 30 per cent or more of the cells may be myeloblasts, or stem cells. In chronic types a small proportion of cells may be slightly immature, and an occasional stem cell may be found, in periods of acute exacerbation of chronic leucemia, extensive immaturity and hemorrhagic features may be present.

ACUTE MONOCYTIC LEUCEMIA WITH HEMORRHAGIC FEATURES

An examination of the blood, made October 21, 1931, revealed the following

Hemoglobin	4.7 gm (28 per cent)
Erythrocytes	1,400,000 in 1 c mm of blood
Leucocytes	6,600 in 1 c mm of blood
Lymphocytes	14.0 per cent
Monocytes	11.0 per cent
Immature monocytes	36.0 per cent
Neutrophils	24.0 per cent
Eosinophiles	5.0 per cent
Basophiles	3.0 per cent
Promyelocytes	2.0 per cent
Leucoblasts	2.0 per cent
Stem cells	3.0 per cent
Normoblasts	13 seen
Platelets	70,000 in 1 c mm of blood
Reticulated erythrocytes	2.6 per cent
Bleeding time	24 minutes
Clot retraction	Absent in 5 hours
Coagulation time of venous blood	5 minutes

Comment on blood picture. Definite monocytosis, with many immature monocytes, including even stem cells. Also, immaturity in the myeloid line, with intermediate forms present, which might also be traced to the stem cell. Myeloid cells probably due to secondary stimulation.

Monocytic leucemia is a third form of leucemia which has come under discussion especially in the last few years. The diagnosis depends almost entirely on careful study of blood smears. This blood count not only emphasizes the importance of study of the morphology of the blood in connection with the diagnosis of acute leucemia, but also demonstrates a case in which the leucocyte count is normal and in which hemorrhagic features are marked. Results of the various coagulation tests are identical with those of acute aplastic anemia in the hemorrhagic phase, and of thrombocytopenic purpura, similar changes in coagulation are seen in arsenobenzol purpura in acute exacerbations or chronic leuce-

mia, and following excessive roentgen therapy, diagnostic distinction from acute leucemia, then, depends on the morphologic characteristics of the cells

AGRANULOCYTIC ANGINA

An examination of blood, April 2 1928, revealed the following

Hemoglobin	55 per cent (Dire)
Erythrocytes	3,650,000 in 1 c mm of blood
Color index	0.7+
Leucocytes	1,100 in 1 c mm of blood
Lymphocytes	92.5 per cent
Monocytes	2.0 per cent
Neutrophiles	4.5 per cent
Basophiles	1.0 per cent

These results give evidence of moderate anemia, it should be noted that the anemia is not extreme. The outstanding feature, however, is the leucopenia associated with a very much reduced absolute number of neutrophiles, and a decreased relative percentage of neutrophiles. The percentage of lymphocytes is high, but the absolute number of lymphocytes is reduced and immature lymphocytes are not present. The absence of severe anemia is evidence against the diagnosis of acute aplastic anemia. On careful analysis, therefore, this blood count alone is very suggestive of agranulocytosis.

In morphologic study of the films in cases of agranulocytosis and hypogranulocytosis few changes from the normal morphologic structure of the lymphocytes, monocytes and neutrophiles are as a rule, to be noted. However, the neutrophiles are frequently "toxic" in appearance, with heavy granulation and distorted nuclei, neutrophiles are not infrequently absent.

HODGKIN'S DISEASE

An examination of blood, made October 9, 1931 disclosed the following

Hemoglobin	10.5 gm (63 per cent)
Erythrocytes	4,760,000 in 1 c mm of blood
Leucocytes	4,000 in 1 c mm of blood
Lymphocytes	18.0 per cent
Monocytes	7.0 per cent
Neutrophiles	69.5 per cent
Eosinophiles	5.5 per cent
Basophiles	1.0 per cent
Reticulated erythrocytes	0.8 per cent

Comment on blood picture. Definite monocytosis, with a shift to the right, toxic neutrophils with a shift to the left, and moderate eosinophilia (see last paragraph in section headed "The right and left shift"). These features usually indicate Hodgkin's disease.

Very rarely, Hodgkin's disease may be present without other recognizable clinical manifestations than splenomegaly. Usually the diagnosis in such a case is made only at operation or at necropsy. However, there are certain features in the blood which when present make one at least suspicious of the existence of Hodgkin's disease, these features are monocytosis and eosinophilia.

Morphologically, in this case, eosinophilia was noted in the blood picture, together with monocytosis and a shift of the monocytes to the right, the shift to the right manifests itself by indentation of nuclei. The polymorphonuclear

neutrophiles showed marked evidence of toxicity, in other words, the granulations were coarse, the nuclei deformed, and in the nuclear material there was poor differentiation between chromatin and parachromatin

POLYCYTHEMIA VERA

An examination of the blood, made July 10, 1931, revealed the following

Hemoglobin	19.9 gm in 100 cc (119 per cent)
Erythrocytes	7,300,000 in 1 c mm of blood
Leucocytes	10,800 in 1 c mm of blood
Lymphocytes	11.0 per cent
Monocytes	4.0 per cent
Neutrophiles	83.0 per cent
Eosinophiles	1.0 per cent
Basophiles	1.0 per cent
Normoblasts	1 seen
Reticulated erythrocytes	1.1 per cent
Viscosity	14.8 times that of water
Blood volume	7,229 cc total volume (129 cc for each kilogram of body weight)

Comment on blood picture: Marked piling of erythrocytes and fragility of leucocytes

The blood of polycythemia is considered, especially because the blood count may vary considerably. In fact, the erythrocyte count may be normal or even less than normal, especially if gastrointestinal hemorrhage has recently occurred. In a case of splenomegaly associated with a normal blood count or moderate anemia, a mistaken diagnosis of splenic anemia may be made. In a case of this type, the former history, the former blood counts, and estimations of viscosity of the blood and of blood volume may be necessary to arrive at a correct diagnosis.

In the results presented, the value for hemoglobin is high, and the erythrocyte count is high, with moderate leucocytosis, the number of polymorphonuclear cells is increased, both relatively and absolutely. These features would indicate either extremely active bone marrow, or very much decreased destruction of blood. Normal viscosity, compared with water, is 4.5, and the increased viscosity of 14.8 would be suggestive of polycythemia. Normal blood volume is approximately 90 cc for each kilogram of body weight and the increased blood volume of 129 cc for each kilogram is probably the most important finding with respect to diagnosis.

The existence of relative or secondary polycythemia must also be considered in a case which presents this blood count. Usually a history of asthma, chronic pulmonary fibrosis, or some other evidence of pulmonary obstruction, or of cardiac disease, may be elicited in cases of relative polycythemia showing slight increase in the erythrocyte count, and slight increase in blood volume.

Morphologically there is little abnormal in the blood smears. The erythrocytes on account of their increased number usually are abnormally "piled up" but if smears are made very thin, this characteristic may not be evident.

SPLENOMEGALY OF INFANCY AND CHILDHOOD

These conditions may conform to well recognized syndromes, such as those of splenic anemia, hemolytic icterus, hemorrhagic purpura, and syphilitic spleno-

megaly There is, however, a large group of cases in which a very peculiar type of blood picture may result from infections, deficient dietary regimen, nutritional disorders, and metabolic diseases. It must be remembered that the blood of infants reacts differently from that of adults and rather readily reverts to the fetal type.

In the syndrome known as von Jaksch's disease, there is anemia with low color index, leucocytosis usually with relative and absolute lymphocytosis, small numbers of myelocytes and metamyelocytes in the peripheral blood, and, in some cases, a large number of immature erythrocytes. None of these features, however, is constant, and the syndrome may be a secondary manifestation. In the anemia of infancy, immature cells are very frequently present in the blood smears, and too much stress cannot be laid on this finding, one must guard against a too hasty diagnosis of leucemia. Probably the most important consideration in studying blood smears of infancy is to decide on the evidence for or against active regeneration on the part of the bone marrow, and it must be constantly kept in mind that many of the morphologic features may be explained by assuming a reversion to the fetal type of production of blood.

MISCELLANEOUS DISEASES

Pernicious anemia occasionally is accompanied by moderate splenomegaly. The spleen is palpable at some time during the course of the disease in most of the cases. The features of the blood count in pernicious anemia are so generally familiar that it is unnecessary to comment on them. Morphologic characteristics of the blood film are macrocytosis, with a fair proportion of oval macrocytes and a shift to the right of polymorphonuclear leucocytes, that is, a higher proportion than normal of many lobed nuclei, connected by fine strands. It may be emphasized that poikilocytosis, ordinarily described as a constant feature, although frequently present may be almost entirely absent.

Eosinophilic hyperleucocytosis with splenomegaly is a rare clinical syndrome. In this condition the eosinophilic polymorphonuclear leucocytes may be increased in number, both relatively and absolutely, to an extreme degree. The leucocyte count is not infrequently high, and it has been suspected that cases of this type may in reality represent a peculiar form of leucemia, however, it is much more likely that the eosinophilia is an unusual reaction to chronic recurring infection.

Lymphosarcoma, it has frequently been thought may be suspected in the presence of suggestive clinical findings when the differential count discloses relative and absolute increase of lymphocytes, with a normal leucocyte count or slight leucocytosis. This suspicion is sometimes corroborated on pathologic examination of the spleen after splenectomy, but just as frequently there is no evidence of lymphocytic hyperplasia in the spleen when the blood count has revealed the features mentioned.

Sickle cell anemia is a very interesting syndrome, and is essentially a form of hemolytic anemia. Sickle cells are frequently found in the blood of negroes without anemia. The erythrocytes assume the sickle-shaped contour in fresh wet preparations. In sickle cell anemia the spleen may be considerably enlarged and the clinical syndrome may be suggestive of hemolytic icterus. Fragility of the erythrocytes, however, is not increased. The spleen not infrequently becomes atrophied later in the disease. Morphologically, aside from the sickle-

shaped erythrocytes, leucocytosis is usually present, and a few immature leucocytes may be found

Abscess of the spleen has, in some instances, been accompanied by an extreme degree of polymorphonuclear leucocytosis. Leucocytes may number as high as 50,000 in each cubic millimeter of blood

Marble bone disease, an affection in which there is increased density of the bones, with encroachment on the marrow cavity, usually reveals a blood count that is slightly suggestive of myelogenous leucemia. In the later phases of the disease the leucemic type of blood picture may become more marked

Subacute bacterial endocarditis occasionally causes the appearance of large reticular cells in the blood smears made from the ear






						
Normal		10	25	47	16	2
Right		9	23	34	24	10
Left	5	30	37	20	8	

Fig. 1—Lobation of nucleus of the neutrophile with percentage of cells found in the normal in a left shift and in a right shift. Taken from Watkins C. H. and Heck F. J. *The Practical Value of Examination of Blood Smears* Minnesota Med. 13 860-864 1930 (Their normal values were taken from Cooke W. E. *Further Observations on the Macrophocyte* Brit. M. J. 1 800-804 1929)

THE RIGHT AND LEFT SHIFT

In view of the consideration of the right and left shift of the neutrophile in the various diseases, Fig. 1 is presented. It will be seen that in normal counts approximately half the cells have nuclei of three lobes, whereas with a shift either to the right or to the left, one third or less of the cells will have nuclei of three lobes. With a shift to the right, a high percentage of polymorphonuclear cells will have five lobes or more. Occasionally a cell is seen containing eight or nine lobes. With a shift to the left, two thirds of the cells may have either crescentic nuclei or two lobes. Many lobed nuclei are most frequently seen in the blood of patients with pernicious anemia and sometimes this feature may be observed even when the blood count is normal, in pernicious anemia with nuclei of many lobes, the lobes frequently are narrow and smooth in contour, and the interlobar strands are elongated. In severe infections, the shift to the left may be extreme, metamyelocytes may be numerous and even myelocytes may be present.

A definite shift is also frequently noted in the maturity of the monocyte. Normally about half the monocytes present indented nuclei. With a shift to the right a majority of the cells will have indented nuclei. With a shift to the left a majority of the nuclei are circular or oval in appearance, and may contain heavy, 'toxic' granulations.

SUMMARY

A series of blood counts, differential counts, and results of the morphologic examination of blood pictures has been presented in diseases with slight, moderate, or marked splenomegaly with the purpose of demonstrating the various features of the blood which frequently are of value in diagnosis. Careful analysis of the characteristics of the anemia, the features of the differential count, the presence of immature cells, the percentage of reticulated erythrocytes, and other evidences of active and inactive regeneration of blood, and the characteristics of the cells on study of the blood film, not infrequently lead to important inferences with respect not only to the diagnosis but also to the prognosis, and to more accurate estimation of the probable results of various methods of treatment. My plea is for more careful analysis of a very common laboratory examination, the results of which too frequently are considered in a superficial manner. The physician can easily become accustomed to examination of blood smears personally, with immediate profit to himself and ultimate benefit to the patient. Many blood pictures are quickly recognized; others, however, require repeated examinations of the blood film before one can be reasonably satisfied.

THE BLOOD PICTURE IN HYPERTHYROIDISM AND IN HYPOTHYROIDISM*

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THE report presented here is based on a study of 1200 routine blood counts made in consecutive cases of hyperthyroidism. Differential counts were made in 250 of these cases.

The report includes routine counts made in 17 cases in which death followed uncomplicated hyperthyroidism and in 51 cases in which postoperative hypothyroidism occurred.

In addition a number of smaller groups are reported in which blood counts were made by one of us (J. H. D.) in a more careful and more detailed manner. These groups include (1) twenty unselected cases of hyperthyroidism, (2) twelve cases in which counts were made before and after thyroidectomy, (3) twenty cases of postoperative hypothyroidism, and (4) ten cases of hyperthyroidism and ten cases of hypothyroidism in which filament, nonfilament counts were made.

REVIEW OF THE LITERATURE

The literature regarding the blood findings in thyroid disorders shows a marked variability in the results and in the conclusions drawn from them. For many years investigators have sought to establish a definite and constant picture whereby they could be aided in the diagnosis and prognosis in cases of hyperthyroidism and hypothyroidism. In 1885, Horsley¹ stated that "marked anemia followed the loss of the thyroid gland." Since that time findings have been reported that run the gamut from marked anemia to no anemia, from lymphocytosis to no lymphocytosis, and from leucopenia to leucocytosis. The conclusions drawn from these varied findings have shown a similar variation, consequently it is difficult for one to reach

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any definite conclusions from the literature, and it was this fact which prompted us to make the investigation here reported

Some writers have reported normal blood findings in hypothyroidism^{4 5} while others report a mild anemia^{6 7 8 9 11 12} and still others report red cell counts varying from 3,000,000 to 4,500,000^{11 12 13 14 15} and lower^{17 18} Falta²⁰ and others²² mention hemoglobin estimations below 60 per cent in cases of hypothyroidism and Howard²³ reports cases with a hemoglobin of 75 per cent

Certain writers have mentioned that in hypothyroidism leucocytosis is present^{2 11 12} Normal white cell counts^{16 22} and leucopenia^{7 12 14 21} are also reported Schermann³ reports that in rabbits a marked reduction in the number of white blood cells follows thyroidectomy

Kocher²¹ found the eosinophile count to be normal in hypothyroidism, but a few believe it to be increased^{1 21 22}

Emery^{22 23 24} states that in myxedema the blood picture is not constant and mentions reduced hemoglobin, a normal white cell count, a decrease in the polymorphonuclear count and perhaps relative or absolute lymphocytosis as frequent findings

Plummer²⁵ reported the blood counts in a much larger group of cases of hyperthyroidism than had been reported before and showed that there was a tendency to lymphocytosis, that a relatively low polymorphonuclear count was a large factor in the production of this change and that the degree of lymphocytosis was a poor index as to the degree or duration of the disease

The statements regarding the blood findings in hyperthyroidism are also quite varied Many writers believe that there is a characteristic blood picture in Basedow's disease^{27 28 31 32 33} which is considered of definite diagnostic importance Some have declared that the lymphocytosis in hyperthyroidism parallels the severity of the disease³⁰ Roth²⁷ believes that the reduced hemoglobin, leucopenia, and lymphocytosis in hyperthyroidism is of prognostic as well as diagnostic value Others disagree with these views and state that there is no characteristic blood picture in hyperthyroidism^{29 32 33 34} and that the lymphocyte count is not in proportion to the severity of the disease³⁵

RESULTS OF BLOOD COUNTS

The average counts for the whole group of 1200 routine blood counts made in 1200 consecutive cases of hyperthyroidism were as follows

Hemoglobin	82.5 per cent
Erythrocytes	4,555,154
Leucocytes	7,034

For the 250 cases in which differential counts were done the results were as follows

Leucocytes	7,954.7
Polymorphonuclears (relative)	62.21
Polymorphonuclears (absolute)	4,948.7
Eosinophils (relative)	0.49
Eosinophiles (absolute)	39.4
Basophiles (relative)	0.09
Basophiles (absolute)	7.5
Lymphocytes (relative)	30.3
Lymphocytes (absolute)	2,412.3
Monocytes (relative)	5.7
Monocytes (absolute)	460.5
Transitionals (relative)	0.90
Transitionals (absolute)	75.5

From the above results it is seen that the red and white cell counts are normal There is an absolute and relative lymphocytosis both of large and small cells There is a relative decrease and a slight absolute decrease in the number of polymorphonuclear neutrophiles and to a less degree in the number of eosinophiles or basophiles There is a slight reduction of hemoglobin

In order to study the relationship between the polymorphonuclear and lymphocyte counts two smaller groups were arranged. The 50 counts in which the number of leucocytes was greatest were grouped together. The average count was as follows:

Leucocytes	11,974
Polymorphonuclears (relative)	68.7
Polymorphonuclears (absolute)	8,232.4
Lymphocytes (relative)	26.0
Lymphocytes (absolute)	3,113.8
Monocytes (relative)	4.5
Monocytes (absolute)	539.8

If 5000 is considered an approximate average neutrophile count it will be seen that the polymorphonuclears have increased in number by more than 3000 cells. On the other hand, the lymphocyte count the normal average of which is in the neighborhood of 1500, has been increased by only slightly over 1600 cells. In this group, then, the absolute lymphocyte count is actually greater than the average for the total series. The increase in the number of polymorphonuclears, however, is so great that the relative lymphocytosis becomes less than the average lymphocytosis for the whole group.

Compare the above figures with those of the averages for a group composed of the 50 lowest white cell counts:

Leucocytes	4,985
Polymorphonuclears (relative)	57.8
Polymorphonuclears (absolute)	2,861.3
Lymphocytes (relative)	36.4
Lymphocytes (absolute)	1,817.5
Monocytes (relative)	4.7
Monocytes (absolute)	234.8

In this group the facts pointed out above are emphasized. The number of polymorphonuclears has fallen to more than 2000 below the normal average, while the absolute number of lymphocytes is still maintained at a figure definitely above the normal. Obviously then in hyperthyroidism the blood counts in cases in which leucopenia is present will demonstrate a greater lymphocytosis than in cases in which no leucopenia is present. This fact is accounted for by the relatively greater fluctuation of the polymorphonuclear cells.

Since lymphocytosis has been shown to be more common when hyperthyroidism is present than in the normal individual, it must have some diagnostic value. The frequency with which lymphocytosis may be present in hyperthyroidism is indicated in Table I representing a total of 266 cases.

TABLE I

RELATIVE LYMPHOCYTE COUNT	NUMBER OF CASES	RELATIVE NUMBER OF CASES
PER CENT		PER CENT
0-15	12	4.5
15-19	13	4.8
20-24	33	12.4
25-29	48	18.0
30-39	84	31.5
40 and above	76	28.5

In 78 per cent of 266 cases the relative lymphocyte count was 25 per cent or above. In 60 per cent of the cases it was 30 per cent or above.

Let us now examine a few groups of cases in which the average height of the basal metabolic rate varies greatly in order to determine whether or not the degree of lymphocytosis is directly related to the severity of the disease.

The first group comprises 36 cases of mild hyperthyroidism. The basal metabolic rate in these cases varied between plus 18 per cent and plus 30 per cent. This group does not include those cases in which the metabolism was below plus 18 per cent, because it was felt that below this level there was a greater possibility of error in the diagnosis. It was thought that a metabolic rate of not more than 30 per cent would be the best index in selecting mild cases. The duration of the disease was not considered. The clinical diagnosis was that of hyperthyroidism in each instance. The blood was not taken during fasting but was taken in all cases within twenty-four hours of the basal metabolism estimation.

Leucocytes	8574
Polymorphonuclears (relative)	68.1
Polymorphonuclears (absolute)	5836
Lymphocytes (relative)	26.3
Lymphocytes (absolute)	2257
Monocytes (relative)	4.6
Monocytes (absolute)	398
Transitionals (relative)	0.9
Transitionals (absolute)	88

Here we see a total white count which is somewhat above the average. The absolute number of lymphocytes is raised more as compared with the polymorphonuclears. There is, however, no lymphocytosis.

The next group is composed of 36 cases of moderate and severe hyperthyroidism. The basal metabolic rate in these cases was plus 40 per cent and above. No cases with any outstanding infection or complication were included. The blood counts were not made on fasting blood and the blood was taken on the day preceding the basal metabolism estimation.

Leucocytes	7207
Polymorphonuclears (relative)	62.1
Polymorphonuclears (absolute)	4478.6
Lymphocytes (relative)	31.5
Lymphocytes (absolute)	2271
Monocytes (relative)	5.3
Monocytes (absolute)	386
Transitionals (relative)	0.9
Transitionals (absolute)	71

On comparing the findings in these severe cases with those in the previous group, we see that the absolute lymphocyte count is very nearly the same as that found in the mild cases. In this group, however, there is a much lower polymorphonuclear count, so that there is a lower total leucocyte count and a mild relative lymphocytosis.

The third group is composed of 17 cases in which death resulted from hyperthyroidism before operative interference was attempted. This group includes no cases in which a complication was a contributing factor, except in the case of those changes which appear immediately preceding death. The blood counts were made from one to seven days before death.

Leucocytes	9491
Polymorphonuclears (relative)	73.0
Polymorphonuclears (absolute)	6861.8
Lymphocytes (relative)	19.4
Lymphocytes (absolute)	1828.3
Monocytes (relative)	6.8
Monocytes (absolute)	649.2
Transitionals (relative)	0.4
Transitionals (absolute)	45.4

Here again the low relative lymphocyte value is not so much due to any great change in the absolute number of lymphocytes as to the marked increase in the number of polymorphonuclears.

The fourth group comprises cases of postoperative hypothyroidism. Fifty-one cases are included in which the basal metabolic rate varied between minus 15 per cent and minus 30 per cent the average rate being minus 23.2 per cent. The blood counts in these cases were made on fasting blood on the morning of the metabolism test.

Leucocytes	8333
Polymorphonuclears (relative)	62.0
Polymorphonuclears (absolute)	5168.6
Lymphocytes (relative)	29.9
Lymphocytes (absolute)	2495.1
Monocytes (relative)	6.9
Monocytes (absolute)	575.4
Transitionals (relative)	0.2
Transitionals (absolute)	21.6

The same comment may be made here as in the case of the previous groups. It appears to be obvious, then, that the relative lymphocytosis bears a very close relationship to the total white count and especially to the absolute number of polymorphonuclears present. It bears little or no relationship to the severity of the disease.

Let us now examine a group of twenty-eight cases, which was arranged in an attempt to determine whether or not the relative lymphocyte count bore any definite relationship to the degrees of hyperthyroidism in individual cases. Cases were selected in which after one night's bed rest in the hospital the basal metabolic rate was in excess of plus 40 per cent.

In these cases a blood count was made on fasting blood immediately following the metabolism test. Five days after thyroidectomy, if the wound had healed per primam, the basal metabolism test and blood count were repeated as before. The results are summarized in Table II which represents the average heights of metabolism and average blood counts on admission and five days postoperatively.

The relative number of lymphocytes showed an average decrease postoperatively but there was a very slight decrease in the absolute number. There was a definite rise in the absolute and relative number of neutrophils.

TABLE II

	PREOPERATIVELY	POSTOPERATIVELY
Basal Metabolism	56.4	10.9
Erythrocytes	4,540,000	4,408,000
Leucocytes	6,730	7,460
Polymorphonuclears (relative)	63.3	70.2
Polymorphonuclears (absolute)	4,253	5,236
Lymphocytes (relative)	33.4	26.0
Lymphocytes (absolute)	2,247	2,039

TABLE III
BLOOD COUNTS IN TWENTY CASES OF HYPERTHYROIDISM

NO	BASAL METABOLISM	PACKED CELLS PER CENT OF NORMAL	RED BLOOD CELL COUNT	HEMO- GLOBIN	VOLUME INDEX	COLOR INDEX	SAURA- TION INDEX	INITIAL WHITE BLOOD CELL COUNT	INITIAL DIFFERENTIAL SMAI				BASO- PHILES
									TOTAL MORPHO NUCLEARS	Lympho cytes	MONOCYTES	EOSINO PHILES	
1	+45	91	4,810,000	84	0.94	0.87	0.92	6,400	59	31	4.5	2.5	1
2	+9	95	4,000,000	80	1.1	1.0	0.84	7,350	43	48	6	3	0
3	+39	97	4,870,000	78	0.88	0.70	0.80	5,350	60.5	35	2.5	2	0
4	+53	73	4,580,000	78	0.85	0.91	1.0	5,800	68	30	1.5	0.5	0
5	+45	84	4,700,000	78	0.89	0.82	0.92	4,700	37	58.5	2.5	2	0
6	+44	93	5,370,000	84	0.93	0.78	0.9	4,800	32	55	10	3	0
7	+36	73	3,850,000	71	0.94	0.92	0.97	6,500	51	46.5	2	1.5	0
8	+64	75	3,040,000	68	1.2	1.1	0.9	4,900	59	35	3	3	0
9	+49	91	4,970,000	84	0.91	0.84	0.92	3,850	47	44	7	2	0
10	+19	82	4,820,000	78	0.85	0.81	0.95	4,850	54	33	7	6	0
11	+65	93	5,190,000	91	0.9	0.88	0.99	8,300	53.5	32	7	7.5	0
12	+21	93	4,680,000	84	1.0	0.9	0.9	6,600	63	24	10	3	0
13	+62	84	4,510,000	84	1.0	0.93	0.92	6,500	37.5	46.5	11	3.5	1.5
14	+24	71	2,880,000	65	1.2	1.1	0.91	4,110	51	38.5	7	3.5	0
15	+3	95	4,940,000	80	0.96	0.81	0.83	3,700	58.5	30	10	1.5	0
16	+71	91	4,990,000	80	0.91	0.80	0.87	5,450	48	40	10	2	0
17	+64	82	5,120,000	78	0.82	0.78	0.95	12,450	68.5	23	6.5	1	1
18	+46	66	3,860,000	78	0.85	0.75	0.87	6,350	56	34	8.5	1.5	0
19	+34	74	4,360,000	65	0.87	0.74	0.89	7,100	39.5	47	9.5	4	0
20	+9	88	4,730,000	78	0.93	0.82	0.88	7,500	41.5	46.5	8.5	3	0.5

Case 2.—Patient has hyperthyroidism clinically exophthalmos and all external eye signs loss of weight, history of plus 35 basal metabolism rate a short time before coming to the Clinic

Case 16.—Hypothyroidism clinically Patient had two x-ray treatments four weeks before admission

Case 20.—Hyperthyroidism clinically Patient has had no iodine treatment

The relative number of lymphocytes was decreased postoperatively in 22, 78.5 per cent, of the 28 cases. Thus the relative number of lymphocytes may serve as some guide to improvement in single cases.

Since the foregoing data have all been based on blood counts made in a routine manner, it was felt that it would be interesting to check these results against a much smaller series made in a more painstaking way by the same individual each time.

The following data are based on such counts made by one of us (J. H. D.). The first group is composed of 20 unselected cases of hyperthyroidism. The counts were all made at about the same time of day and with the same instruments each time. An analysis of the findings in this group gave the following averages:

The average basal metabolism was plus 42.7 per cent.

Leucocytes	6,710
Lymphocytes (relative)	38.5
Lymphocytes (absolute)	2,343
Polymorphonuclears (relative)	51.2
Polymorphonuclears (absolute)	3,435
Erythrocytes	4,497,000
Hemoglobin	77.3
(Haden-Hausser instrument)	
Volume index	0.94
Color index	0.86
Saturation index	0.9

In 55 per cent of the cases the hemoglobin was below 80 per cent.

In seven cases the volume index was below 0.9, in three cases it was above 1.

In 13 cases, 65 per cent, the color index was below 0.9, in two cases it was below 0.8, and in two cases it was above 1.

In seven cases, 35 per cent, the saturation index was below 0.9.

A consideration of the initial differential counts in this group shows that in 18 or 90 per cent a relative lymphocytosis of 30 per cent or above was present, and that in 45 per cent a relative lymphocytosis of above 40 per cent was present. Of the latter group, in seven there was an absolute increase in the number of lymphocytes as compared with the normal count and as compared with the average absolute count of the series (2343). Of these seven cases in which a relative lymphocytosis of 40 per cent or above was present in each there was an absolute decrease in the number of polymorphonuclear cells.

In 80 per cent of this series the relative polymorphonuclear count was below 60 per cent. The average absolute polymorphonuclear count was 3435.5 which is 1334 less than the accepted average normal count.

The monocyte, eosinophile, and basophile counts were essentially normal (Table III).

In twelve cases of hyperthyroidism blood counts were made on the admission of the patient to the hospital, and repeated from four days to one and one-half months postoperatively. In all cases the basal metabolism had become greatly reduced between the time of the first and the last blood counts. In six cases there was an increase and in six there was a decrease in the absolute number of lymphocytes. The relative lymphocytosis was reduced in every case, however, obviously because of a comparable rise in the polymorphonuclear count (Table IV).

TABLE IV
RELATIVE AND ABSOLUTE LYMPHOCYTOSIS IN TWELVE CASES OF HYPERTHYROIDISM

NO	BASAL METABOLIC RATE	PREOPERATIVE RELATIVE LYMPHOCYTE COUNT	POSTOPERATIVE RELATIVE LYMPHOCYTE COUNT	PREOPERATIVE ABSOLUTE LYMPHOCYTE COUNT	POSTOPERATIVE ABSOLUTE LYMPHOCYTE COUNT	DAYS POSTOPERATIVE
1	+34	47%	34%	3337	3434	(16 d)
2	+49	44%	40%	1694	1880	(1½ mo)
3	+53	30%	21%	1740	2845	(7 d)
4	+64	35%	28 5%	1715	3947	(4 d)
5	+45	58 5%	32%	2749	3200	(4 d)
6	+44	55%	42%	2640	3297	(27 d)
7	+ 9	48%	24%	3728	1599	(5 d)
8	+36	45 5%	21%	2993	1921	(8 d)
9	+ 9	46 5%	27 5%	3487	2415	(8 d)
10	+46	34%	16 5%	2159	2079	(14 d)
11	+39	35%	23%	1872	1495	(3 d)
12	+65	32%	24 5%	2656	2621	(4 d)

In twenty cases of postoperative hypothyroidism blood counts were made in the same careful manner. The average basal metabolism in 19 cases of this series was minus 23.1 per cent (see Table V). The average counts were as follows:

Leucocytes	6905
Lymphocytes (relative)	36.5
Lymphocytes (absolute)	2453.5
Polymorphonuclears (relative)	56.2
Polymorphonuclears (absolute)	3929.5
Erythrocytes	4,611,700
Volume index	0.95
Color index	0.87
Saturation index	0.88
Eosinophiles, monocytes and basophiles	Normal in all cases
Hemoglobin	80.0

In this group in only three cases was the erythrocyte count below 4,000,000. The hemoglobin average for the series would have been somewhat lower had it not been for one case in which there was a slight polycythemia of 5,690,000 with a hemoglobin of 104 per cent. In 50 per cent of the counts in cases of hypothyroidism the hemoglobin was below 80 per cent.

In this group the average color index, volume index, and saturation index were all slightly below normal as was noted also in the group of cases of hyperthyroidism. In five cases the volume index was below 0.9 and in four it was above 1 (Table V).

In twelve cases the color index was below 0.9.

A filament, nonfilament count was made in ten cases of hypothyroidism and the average number of nonfilamented cells was found to be 19.3. In each of these cases the total leucocyte count was normal. In ten cases of hyperthyroidism the average preoperative nonfilament count was 12.3. In these same cases the average postoperative nonfilament count was 12.1.

In one instance the total white cell count was raised to 13,850 postoperatively and the nonfilament count was 25, in the other cases the white cell counts were all within normal limits.

Comparing the counts obtained in the smaller group with the routine counts,

TABLE V
BLOOD COUNTS IN TWENTY CASES OF HYPOTHYROIDISM

NO	BASAL METABO- LISM	PACKED CELLS PER CENT OF NORMAL	HEMO- GLOBIN	VOLUME INDEX	COLOR INDEX	SATURA- TION INDEX	RED BLOOD CELL COUNT	WHITE BLOOD CELL COUNT	TOLE- MOPLIO NUCLEARS	LYMPHO- CYTES	MONOCYTES	EOSINO- PHILS	BASO- PHILS
1	-28	82	71	1.1	1.0	0.86	3,565,000	3,700	60	35	3	2	
2	-37	88	80	1.0	0.94	0.9	4,280,000	1,850	43	19	6	2	
3	-24	97	84	1.01	0.87	0.86	4,820,000	7,050	11	49	8	2	
4	-25	100	84	1.0	0.84	0.81	5,050,000	5,150	60.5	30	7.5	1	1
5	-16	97	91	1.01	0.94	0.93	4,810,000	9,800	53	31	10	2	
6	+3	84	75	0.84	0.75	0.88	5,000,000	5,650	51	16	2	1	1
7	-37	100	91	1.0	0.93	0.91	4,850,000	8,500	62	30	6	2	
8	-14	100	91	0.9	0.82	0.91	5,560,000	8,200	69	27	1	3	
9	-12	95	84	0.96	0.87	0.86	4,840,000	5,100	53.5	38	5	3	0.5
10	-9	73	58	0.82	0.65	0.69	4,410,000	13,050	63	30	4.5	2	0.5
11	-30	95	91	0.98	0.94	0.95	4,630,000	8,800	55	31	5.5	4	
12	-28	68	71	0.75	0.78	1.0	3,400,000	1,500	62	31.5	0	2.5	1
13	-27	91	78	0.96	0.82	0.85	4,720,000	8,650	56.5	38	1.5	2.5	1.5
14	-31	86	78	1.0	0.95	0.90	4,130,000	6,500	61	31.5	1	1.5	
15	-23	73	65	0.91	0.81	0.89	4,010,000	6,650	61	33	1.5	1.5	
16	-30	88	78	0.88	0.78	0.88	5,160,000	7,400	59	33	5.5	0.5	2
17	-17	84	84	0.85	0.85	1.0	4,910,000	5,550	11.5	51	3	1.5	
18	-19	88	71	1.2	1.0	0.80	3,600,000	6,000	49	11.5	4.5	2	
19	-4	84	78	1.0	0.96	0.92	4,570,000	7,250	60.5	32	5	1.5	1
20	-28	115	104	1.01	0.91	0.9	5,600,000	5,750	59	32.5	6.5	2	

Case 6.—Patient had basal metabolism rate of minus 25 last year and has had thyroid extract intermittently since hypothyroidism. This case is not included in average basal metabolism rate for group. Clinically she has been hypothyroidism. Case 10.—Basal metabolism rate has been minus 22. Patient has had thyroid extract at intervals for three years. Clinically she now has hypothyroidism, marked improvement on thyroid extract. Case 19.—Patient has definite symptoms of hypothyroidism even though basal metabolism rate is only minus 1. Previously she has had marked hypothyroidism with all signs and has improved on thyroid extract.

the findings are approximately the same except that in the former series of cases the total leucocyte count is slightly lower, the relative lymphocyte count is a trifle higher, and the relative polymorphonuclear count is slightly lower

CONCLUSIONS

1 In hyperthyroidism, the red blood cell count is normal but there is a slight reduction of hemoglobin

2 There is a relative lymphocytosis of 30 per cent or above in 60 per cent of the large series of cases of hyperthyroidism and in 90 per cent in the smaller series. The presence of a lymphocytosis is therefore of some diagnostic value

3 The relative lymphocytosis is dependent on two factors in the blood (a) the absolute lymphocyte rise, (b) the total leucocyte count

The total leucocyte count has been found to be variable, the variation being due, chiefly, to the variation in the neutrophiles. There is a greater lymphocytosis, therefore, in the presence of leucopenia

4 In 28 cases examined while the metabolic rate was high, and again after operation, the average lymphocyte count was found to be lowered by operation. In the majority of cases there was a fall in the relative number of lymphocytes present. In the series of 20 cases counted apart from the routine counts 50 per cent of the cases showed an absolute increase of lymphocytes postoperatively (counted from four days to one and one half months postoperatively), and 50 per cent showed an absolute decrease. All showed a relative decrease

5 The degree of lymphocytosis on one estimation is not an index of the severity of the hyperthyroidism

6 The hemoglobin in hypothyroidism is reduced slightly as in hyperthyroidism. The degree of relative lymphocytosis in hypothyroidism is about as high as that in hyperthyroidism

7 The average nonfilament count in ten cases of hypothyroidism is higher than that in ten cases of hyperthyroidism

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CLINICAL AND EXPERIMENTAL

AN ANALYSIS OF THE BLOOD PICTURE IN 100 CASES OF MALIGNANCY*

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THIS study was undertaken to determine, insofar as possible, the presence or absence of a definite blood picture in malignant disease, and if such is present, to determine whether it is constant enough to be of clinical value. One hundred consecutive cases were chosen for this purpose (Table I). The diagnoses of malignancy were confirmed by biopsy, autopsy, x-ray, or surgical exploration. The hematologic observations comprised

- a Bleeding time and clot retraction time
- b Tourniquet test
- c Platelet count
- d Coagulation time (capillary pipette method)
- e Hemoglobin percentage (Dare)
- f Erythrocyte count
- g Color index
- h Leucocyte count
- i Differential count
- j Red cell morphology including reticulation
- k Fragility test

In the differential study, special attention was paid to the neutrophils, the relation of the number of band forms and the presence or absence of eosinophiles.

Bleeding Time and Clot Retraction Time—The bleeding time was carried out by Duke's method. Caution was observed in an effort to prevent undue laceration of the skin and underlying vascular structures. The normals observed in our laboratory, varied from one to three minutes. A bleeding time therefore, of over three minutes was considered abnormal.

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This test was done on 73 cases. From the standpoint of duration of bleeding time, the cases can be classified into three groups. The first (bleeding time between five and thirty seconds), consisted of 39 cases or 55 per cent of the total in this series. The second (bleeding time between thirty-one and fifty-nine seconds), consisted of 10 cases, or 7 per cent. The third group

TABLE I
CLASSIFICATION OF CASES STUDIED

Carcinoma of stomach	22 cases
Carcinoma of colon	15 cases
Carcinoma of pancreas	6 cases
Carcinoma of gall bladder	4 cases
Carcinoma of tongue	2 cases
Epithelioma of lip	1 case
Carcinoma of lung	15 cases
Carcinoma of breast	8 cases
Carcinoma of uterus	2 cases
Carcinoma of ovary	3 cases
Carcinoma of prostate	1 case
Carcinoma of bladder	1 case
Adenocarcinoma of urethra	1 case
Hypernephroma of left kidney	1 case
Adenocarcinoma of right kidney	1 case
Lymphosarcoma	7 cases
Sarcoma (including retroperitoneal)	5 cases
Carcinoma of tonsil	1 case
Carcinoma of thyroid	1 case
Metastatic carcinoma of bones	3 cases

(bleeding time one minute or over), numbered 24 cases or 33 per cent. In the latter group, was a case of cervical lymphosarcoma in which the bleeding time was four minutes (Case 63). Thus, the observations of Bock and Rausche¹ who could find no special alterations in the bleeding time in their series of 13 cases of carcinoma were not confirmed. It is significant, also, that two-thirds of malignant cases presented a bleeding time of less than one minute, and that a prolonged bleeding time such as cited by Rawitsch and Waischawskaja - was not observed.

The etiologic factors responsible for the lack of bleeding tendency in malignant conditions are as follows: increased or normal number of platelets, leucocytosis, and good clot retraction (determined by capillary method) all of which were revealed in this study. In none of the cases was there present a deficient clot retraction. This observation is confirmed by the experimental work of Van Allen² who in a study of the relationship between clot retraction time and tumors in rabbits found an increased clot retraction rate as the tumors grew and a normal rate as the tumors receded.

Tourniquet Test—The tourniquet test in malignancy is almost always negative in the 100 cases studied. The test was performed as follows: Pressure ranging between 100 and 120 mm. of mercury was applied about the arm as in taking a blood pressure reading. After three minutes the cuff was removed. A reaction was considered positive when there were present petechial spots about the bend of the elbow anteriorly or posteriorly. In but one case of this series, a patient with carcinoma of the prostate with metastasis to the rectosigmoid (Case 66), was there a "one-plus" reaction (1-5 petechiae). The

bleeding time in this case was forty-five seconds, coagulation time seven minutes and twenty seconds the platelet count 250,000 per c mm. In all the other cases tourniquet test was negative.

It is of interest to point out in this connection, that in one instance, a patient with carcinoma of the lung (Case 98) presented purpuric spots over his chest and abdomen. On necropsy, widespread metastasis into many organs including the bone marrow, were demonstrated. The platelet count was 250,000 per c mm. This lesion may have been a preagonal event as indicated by Hayem¹ or part of the picture of cardiac decompensation which the patient presented, giving rise to increased capillary permeability. The tourniquet test was negative. Rawitsch and Waischawskaja² report a markedly positive Rumpel Leede phenomenon together with a thrombocytopenia (platelets 30,000 per c mm and increased bleeding time of seventeen minutes) in one case.

Platelets—Thrombocytes were estimated in 80 cases by the sodium citrate method of Ottenberg and Rosenthal. In but two instances, the platelet count was less than 150,000 per c mm. Three cases showed counts above 500,000 per c mm. The remainder of the series (95 per cent of the cases) showed counts varying from 150,000 to 400,000 per c mm. These observations indicate that thrombocytopenia is uncommon in malignancy. In one case of carcinoma of the stomach (Case 29) a count of 140,000 platelets per c mm were found, this patient also presented a leucopenia (5800) and a hemoglobin of 32 per cent. In another instance (Case 37), a patient with sarcoma of the right arm, showed a platelet count of 136,000 per c mm on admission. One month later the thrombocytes dropped to 60,000 per c mm. This phenomenon may have been due to x-ray treatment given one month previous to admission or to the progressiveness of the neoplasia with a consequent effect on the megakaryocytes of the bone marrow. A search of the literature reveals but rare instances in which thrombocytopenia was an accompaniment of malignancy. Johann Cohen³ reports a case of metastatic carcinoma of the bone marrow with persistent thrombocytopenia. Similar cases have been described by other authors.⁴⁻¹⁴ These cases, however, are exceedingly rare. Naegeli¹⁵ states that the platelet count in carcinoma is always high. Rud¹⁶ has made similar observations. Hayem¹ found that the platelets diminished preagonally. In this series of cases a preagonal decrease of platelets was not found.

Thrombocytosis as indicated above, is by far the most outstanding finding in malignancy. The marked tendency to nonmalignant thrombosis in carcinoma is ascribed by Niegeli¹ to this factor. In our series, three cases stand out prominently in the intensity of the platelet response. In a case of adenocarcinoma of the transverse colon (Case 75) there was a platelet count of 510,000 per c mm. In the second case one of early carcinoma of the breast (Case 77), and in the third case one of carcinoma of the colon (Case 94) there were 720,000 platelets per c mm.

Rud¹⁶ in a study of 40 cases of cervical carcinoma found an average count of 463,000 per c mm. In half of the cases it was above 450,000 per c mm, in the others it ranged between 280,000 and 450,000 per c mm, the maximum being 812,000 per c mm. He believes that the factor which causes the anemia

contributes to the cause of the thrombocytosis and that the latter tends to increase with the gravity of the lesion. One cannot agree with this dictum, for in the three cases in this series, cited as having the highest platelet counts, none showed evidence of metastasis or other criteria of marked invasiveness.

Coagulation Time—The coagulation time was determined in 71 cases and was found to be within normal limits or only slightly reduced. The normals varied from four to ten minutes. In only 11 cases was the coagulation time over seven minutes. The longest coagulation time was nine minutes. This was in a case of perforating carcinoma of the stomach (Case 81). The average coagulation time was approximately four and one-half minutes. Boek and Rausche¹ were unable to find significant changes in their study. Rud¹⁷ in a study of 40 cases of cervical carcinoma, found a constant and considerable reduction in coagulation time. This series does not present evidence of marked acceleration of coagulation, and it may be concluded that there is no deficiency in coagulation.

The tendency to increased or normal coagulability of the blood of individuals suffering from malignant disease, may be ascribed to the normal resistance of the platelets as well as to their increased number, the latter liberally supplying thromboplastic substance. In a similar way, leucocytosis plays a prominent part as a source of available factors necessary in the process of coagulation. An increase of blood fibrinogen may be another factor aiding coagulation, as was pointed out by Rud.¹⁷

It may be of interest to point out at this time, that radium therapy favors rapid coagulation. This observation was made by Paimov¹⁸ in a study of 40 cases of carcinoma of the uterus, following radium therapy.

Hemoglobin—The hemoglobin estimation (Dare) was made in 99 cases, which were divided arbitrarily into three groups. Group 1 (Hb 50 per cent or below) made up 43 per cent of the cases, Group 2 (Hb 51 to 64 per cent) 25 per cent of the cases, Group 3 (Hb 65 per cent or above) 32 per cent of the cases.

The hemoglobin in malignancy, therefore, presents two extremes. The lowest readings which were found in about half of the cases were most frequently met with in malignancy of the gastrointestinal tract, gastric involvement yielding an average of 38 per cent hemoglobin, and carcinoma of the colon, an average of 50 per cent hemoglobin. The higher readings were obtained in pulmonary and breast malignancies, the former showing an average of 67 per cent and the latter 69 per cent hemoglobin. The sarcomas had a lower range of hemoglobin than that shown in the cases of pulmonary and breast neoplasm, averaging approximately 56 per cent.

As pointed out above, 16 cases in Group 3 showed a range of hemoglobin between 75 to 95 per cent. It may be useful to enumerate the instances in which these findings were obtained even though they be isolated cases. Carcinoma of the lung (5 cases), of the breast (4 cases), of the stomach (2 cases), of ovary (1 case), of tongue (1 case), adenocarcinoma of the urethra (1 case), carcinoma of the rectum (1 case), and of tonsils (1 case). With esophageal involvement the blood picture of carcinoma of the stomach is altered by a tendency to erythrocytosis and increase in hemoglobin. Deficient fluid in-

take in these cases may be responsible for the relative polycythemia and by the same token a relative hemoglobin increase¹⁹ due to blood concentration. The hemoglobin and red cell estimations in these cases bear out this point. Case 17 had a hemoglobin of 80 and an erythrocyte count of 5,600,000 per c mm. Here esophageal involvement was suggestive but not demonstrable by x-ray. Case 35 showed a hemoglobin of 79 per cent and a red cell count of 4,950,000 per c mm. In this instance, esophageal involvement was demonstrable. None of the other cases in the gastric series presented evidence of esophageal involvement. On the other hand, Cabot²⁰ in a study of 72 cases of gastric carcinoma, reported 19 in which the red blood cell counts were 5,000,000 per c mm or over. The esophageal involvement in that series was not discussed but nevertheless may have been present, and not noted.

Another point worthy of note, is the evident disparity between the hemoglobin as compared to the erythrocyte readings. For example, Case 4, a patient with carcinoma of the hepatic flexure of the colon, yielded a hemoglobin of 18 per cent and a red cell count of 4,000,000 per c mm. Similar findings were by no means uncommon in this series, having been found in half the cases. This fact was also observed by Eisen¹⁹ in a study of 353 cases. He attributed the high erythrocyte values in carcinoma of the respiratory passages and the esophagus as possibly due to a masking of the anemia in the first instance by a concentrating effect upon the blood serum of a prolonged interference with water ingestion. He also found a rise in red blood cell and hemoglobin value in patients whose lesions were of longer duration. This finding may be true of malignancy in general but certainly does not hold true for neoplasm of the colon as indicated above. He stressed the fact that hemolysis and aplasia of the bone marrow did not play a part in the production of anemia in cancer. He was convinced that the anemia of malignancy bore no relation to the age of the patient and was unable to find any effect on the anemia by therapeutic measures. The most severe anemias, he asserted, occurred in carcinoma of the stomach. This series corroborates his findings. He further stated that adenocarcinoma was associated with greater average degree of anemia than the sarcoma, colloid, or simple carcinoma, because of the greater tendency to hemorrhage, ulceration and infection, as well as higher average grade of malignancy. He found no definite relationship between the extent of involvement or degree of dissemination and the degree of anemia.

Anemia—A search of the literature presents confusion in the use of the term anemia, especially as applied in the study of malignancy. Some use the term anemia to express a depression in hemoglobin below the normal figures, others to indicate a paucity of erythrocytes, and still others, a low color index. It is apparent therefore, that the term anemia is too general, and frequently ambiguous. For this reason it was found expedient to employ more specific terms, namely erythrocytopenia to indicate a low red cell count and hemoglobinopenia a low hemoglobin value.

The first effect on the red cells in malignancy appears to be a hemoglobinopenia with normal or increased erythrocytic values. The second change if the malignancy progresses is an increase in the hemoglobinopenia with a

beginning erythrocytopenia. If the process lasts long enough, there is a further reduction of the hemoglobin and erythrocytic values. In this last phase there may be an imbalance in the rate of destruction and production of hemoglobin and red cells, tending to increase the color index. In the third phase, the result of imbalance such as mentioned above, may be either a low or high color index. It is this latter group of cases that may offer great difficulty in differentiating malignancy from true Addisonian pernicious anemia.

Anemia in malignancy does not necessarily depend on actual metastasis but may be present even in the absence of the latter. It may be due to another factor such as a specific carcinomatoxin which affects the bone marrow. If one accepts this specific nature of the carcinoma substance as responsible for the changes in the bone marrow, one cannot accept the dictum of Isaacs³ that the presence of anemia in the absence of bleeding suggests metastatic bone marrow involvement. Pincus⁴ states "Although the ordinary anemia of cancer is not dependent upon the presence of metastasis in the bone marrow it is dependent upon changes in that organ. The chronic anemia of protracted cases of carcinoma leads to increase in the amount of red cellular marrow in the bones." One must agree with Pincus⁴ that "The essential feature in carcinoma is the evidence of grave disturbance of the erythropoietic organs while in addition there appears to be some interference of a stimulating nature acting under leucopoietic mechanism." The latter results in the leucocytosis which is a well known concomitant of malignancy and was mentioned by Alexander² as far back as 1878. Contributing factors in the production of anemia are excessive radiation and nutritional disturbances from inability to swallow, retain or digest food.

It has been found that radium treatment increases the hemoglobin¹⁵ but has no influence on the red cells. This fact offers a ready explanation for the presence of a high color index following radium therapy in malignancy.

Erythrocytes.—The erythrocytes were enumerated in 100 cases, the latter divided into three groups. In Group 1 which consisted of 13 per cent of the cases red cells were below 3 000 000 per c mm. In Group 2 64 per cent of the cases, the red cells ranged between 3 to 5,000,000 per c mm. In group 3 23 per cent of the cases the red cells were over 5 000 000 per c mm. It is obvious, therefore that about two-thirds of malignant cases show a slightly depressed or almost normal red cell count (3 to 5 000,000 per c mm) one eighth, a moderately depressed count (below 3 000 000 per c mm) and one-fifth, normal or increased count (over 5 000 000 per c mm).

The lowest counts in Group 1 were in a case of carcinoma of the stomach (Case 51) with 1 440 000 red cells per c mm and in a case of adenocarcinoma of the uterus (Case 1) with 2 000 000 per c mm. All the others in this group were above 2 000 000 per c mm. Cases with less than 1 000 000 cells per c mm are rare according to Naegeli¹. The highest count in Group 3 was found in a carcinoma of the head of the pancreas (Case 91) with 6 220 000 red cells per c mm. Cases with lesions in the stomach yielded the lowest number of red cells (average 3,690 000 per c mm) whereas those with lesions in the

colon, lung, breast, pancreas, and sarcomas yielded the higher figures, the average range being 4 to 5,000,000 red cells per c. mm.

It is interesting to note that higher red cell and hemoglobin values were found in cases with involvement of the left side of the colon, than in those with involvement of the right side. The average count in cases with lesions of the left side of the colon was 4,860,000 red cells per c. mm., while in those of the right side, the average was 3,580,000 red cells per c. mm. The average hemoglobin percentage in cases with lesions of the right side of the colon was 31.5, whereas in left-sided lesions, it was 70. These results are in accord with the findings of Prickman¹ and Alvarez, Judd, MacCarty and Zimmerman.² The latter have studied varying degrees of anemia produced by carcinoma in different parts of the colon in a series of 1168 cases. They concluded that carcinoma of the cecum and ascending colon had a greater tendency to produce anemia than that of the transverse and descending portions. They stated: "The gradation cannot be explained by greater loss of weight, more severe hemorrhage, or differences in malignancy, the essential factor in the production of anemia is the presence of a large ulcerated area from which blood oozes and through which bacteria enter. As the tumors grow, until they cause obstruction, those developing in the regions of the greatest diameter will become largest before the symptoms of obstruction appear. The diameter of the colon decreases from about 6 cm. in the cecum to 2½ cm. in the sigmoid, hence, the greater anemia in carcinoma of the proximal colon."

Color Index—The color index is the weakest link in the chain of hematologic evidence. It must of necessity be of minor diagnostic importance because it depends upon factors which are constantly variable. The estimation of the color index depends upon the estimation of the erythrocyte count, the hemoglobin determination, and the personal equation. The hemoglobin estimation is notoriously unreliable because of the lack of equally standardized hemoglobinometers in general use. The personal equation is a great factor, for it cannot be denied that these findings are more reliable when done by specially trained workers than when performed in a desultory routine fashion.

For these reasons the estimation of the color index loses its value in the differential diagnosis between pernicious and perniciouslike anemias. It therefore becomes necessary to study the morphology of the red cells, i. e., the presence or absence of macrocytes rather than to depend upon the color index alone. The latter becomes of value as confirmatory evidence only when morphologic changes in the red or white cells are definite. That is, when marked anochromasia and hypochromasia are found, a low or even high color index, indicates a secondary anemia. However, a marked macrocytosis points to pernicious anemia despite the fact that the color index does not exceed unity. This is true especially when there are corroborative findings in the rest of the blood picture. This consideration becomes exceedingly important in the blood picture in malignancy. Difficulties are encountered in the differential diagnosis between gastric carcinoma and pernicious anemia.³ These difficulties can be avoided by adopting morphologic criteria as a primary consideration rather than the color index routinely performed. In other words,

the color index should not be regarded as the ultimate dividing line between pernicious, secondary, and perniciouslike anemias. It is noteworthy that out of the 100 cases of this series, 17 showed a high color index and in only one of these was macrocytosis present.

The average color indexes accompanying lesions in the various organs are as follows: colon 0.57, stomach, 0.61, lungs 0.75, breast 0.76, pancreas, 0.72. The lowest color index was 0.22, in a case of carcinoma of the hepatic flexure of the colon (Case 42). The highest color index 1.2 was obtained in a patient with adenocarcinoma of the uterus (Case 1).

Baraduh²⁴ in a study of 81 cases, found one with a color index above one and three with a color index of one. Parmov¹⁵ pointed out that radium therapy tends to raise the color index so that even a low color index may be increased to normal or above. He also called attention to the fact that in carcinoma of the uterus a color index of over 1.8 after radium therapy was a bad prognostic sign.

Red Cell Morphology—In studying the changes in morphology of the red cells in this series, the following conditions were encountered: anisocytosis, poikilocytosis, polychromasia, microcytosis, macrocytosis, anochromasia, hypochromasia, normoblastosis and reticulocytosis. To facilitate recording these changes the following scheme was adopted. In describing anisocytosis for example, the finding of 3 to 5 anisocytic cells per hundred red blood cells was designated as slight or "one-plus," 5 to 10 per hundred red blood cells, moderate or "two-plus," 10 to 15 per hundred red blood cells, marked or "three-plus" and 15 or over per hundred red blood cells, very marked or "four-plus." The other changes, poikilocytosis, polychromasia, etc., were recorded in a similar manner. The normoblast value was determined by noting the number of these cells encountered in doing a differential count of 100 leucocytes.

Anisocytosis—Anisocytosis presented itself in 32 cases or 32 per cent of this series. In 26 cases, the reaction was slight, in 5 moderate and in 1, marked. One-third of the cases in this series showed varying degrees of this change. Eighty-one per cent of the cases that showed anisocytosis, showed it to a slight degree. Anisocytosis then is a relatively uncommon finding in the blood changes in malignancy. An interesting observation is that in 13 instances, anisocytosis was associated with metastases.

Poikilocytosis—Poikilocytosis was encountered in 19 cases or 19 per cent of this series. Slight changes were found in 13 cases. In 5 instances, there was a moderate, and in one instance, a marked poikilocytosis. Metastases were encountered in 4 cases.

Polychromasia—Polychromasia was present in 5 cases of this series, in 4 of which the change was slight, and in one, moderate. No metastases accompanied this change.

Microcytosis—Microcytosis was present in 24 cases or 24 per cent of this series. In 17 cases there was a slight change, in 5 a moderate change and in 2, a marked change. Metastases were associated in 9 instances. Naegeli¹⁰ observes that carcinoma presents a secondary anemia with low color index.

and small pale red blood cells. This finding was not confirmed inasmuch as microcytosis was a finding in only 24 per cent of this series of cases.

Macrocytosis—Macrocytosis was present to a slight degree in only 9 cases of this series. Naegeli¹² has observed macrocytes mostly of the polychromatic variety in marked regeneration although the picture of secondary anemia was still outspoken. He further observed that carcinomas with a high color index accompanied by macrocytes and normoblasts always speaks for bone marrow metastasis. However, metastases were associated in but three instances, in only one case of which (Case 76) could involvement of the bone marrow be demonstrated.

Anochromasia—This term has been defined by Watkins²⁷ as the absence of hemoglobin in the center of the red cells which give them a doughnut appearance, with a relatively rich rim of hemoglobin at the periphery in contradistinction to hypochromasia which designates a general pallor of the entire cell without reference to cell formation. This change was present in greater frequency than any of the others, being present in 52 cases or 52 per cent of this series. There were slight changes in 19 cases, moderate in 18, marked in 14, and very marked in one. It appears from these observations that anochromasia is the most constant morphologic change of the red cells in malignancy. Metastasis was associated with polychromasia in 20 instances.

Hypochromasia—This was found in 33 cases or 33 per cent of this series. The change was slight in 21 cases, moderate in 12 cases, and marked in 3 cases. These changes were associated with metastases in 10 instances.

Normoblastemia—Six cases in this series contained normoblasts in the peripheral blood. They were found in the following instances: Case 1, carcinoma of the stomach with 9 normoblasts per hundred white blood cells, Case 29, carcinoma of the stomach with 1 normoblast per hundred white blood cells, Case 54, lymphosarcoma with 1 normoblast per hundred white blood cells, Case 78, carcinoma of the uterus, and Case 98, carcinoma of the lung with 1 and 5 normoblasts per hundred white blood cells respectively. In this group, bone marrow metastasis was demonstrable in but one case (Case 98). General metastases were present in two instances.

Reticulation—Reticulocytosis was present in 36 cases or 36 per cent of this series. It was slight in 31 instances and moderate in five. A moderate increase in reticulation was found in the following instances: Carcinoma of the breast, 2 cases, carcinoma of the uterus, 1 case, carcinoma of the stomach, 1 case, metastatic carcinoma of the bone, 1 case. Metastases were encountered in 18 instances.

Changes in Red Cell Morphology and Their Relation to Metastasis—Obama²⁸ in a study of sarcoma in rabbits concluded that an increase in the myeloid and normoblastic elements accompanied by anisocytosis and polychromasia, indicated advancement of the lesion. No such conclusion could be drawn from this series. In but two of the cases which presented normoblastemia, was the presence of metastasis demonstrated. However, one cannot deny the observation by Naegeli¹² that high erythroblast counts are associated with signs of bone marrow involvement by carcinoma. Furthermore it has been shown by Naegeli¹²

that metastasis may be present in the bone marrow without concomitant blood changes. This would explain the Indeterminate group of blood pictures observed in this series. Similar cases were observed by Hirschteld.¹⁴ Zadek and Sonnenfeld¹⁵ reported two cases of prostatic carcinoma in which bone metastasis of the osteoplastic variety was found without the presence of normoblasts. There were, however, other changes in the blood indicative of bone marrow metastasis, namely, leucopenia and relative lymphocytosis, corresponding to the infiltrative or destructive groups to be described in this series.

It will be noted from the foregoing, that the frequency of metastasis was a more or less incidental feature in association with changes in red cell morphology. What relationship there exists between metastasis and changes in red cell morphology could not be determined. It appears that metastasis is associated with varying degrees of changes in red cell morphology, the most prominent being reticulocytosis, anisocytosis and anochromasia, the less prominent being microcytosis, and hypochromasia. The study did not reveal any prominent association between normoblastemia, polychromasia and metastases. The reason may be twofold. In the first place, then low incidence in this series (polychromasia was present in 5 per cent and normoblastemia in 6 per cent of this series) does not permit one to draw general conclusions, and secondly, it is possible that although metastases may be present, they may not be demonstrable or relatively scant.

Fragility Test—Mitrovich¹⁶ in an investigation of 23 cases found no significant changes in the resistance of erythrocytes in malignant conditions to hypotonic saline. The test was performed in 5 cases. In two cases hemolysis began at 0.42 and was complete at 0.30. In one case it ranged from 0.44 to 0.34, and another case from 0.42 to 0.32. In the last case, hemolysis began at 0.38 and was complete at 0.30. These results may be considered normal except for the latter instance in which it must be assumed there was a diminished fragility. However, conclusions cannot be drawn from this small series of cases.

Leucocytosis—Leucocytosis was present in 61 per cent of the cases. It may be convenient at this point to define two terms which are used in connection with this study, especially in their relation to leucocytosis. 'Asegmentopenia' may be defined as the absence of an increase in band forms accompanying leucocytosis or more rarely, leucopenia. It is well known that leucocytosis of the infections and other varieties is associated with an increase in band forms (asgmentophilia). In this study the absence of band forms in relation to leucocytosis is conspicuous. This relationship has not previously been described in the literature. Fifty-two cases, or 85 per cent of all cases presenting leucocytosis were associated with asgmentopenia. Forty-three of the cases, or 70 per cent of the cases showing leucocytosis were associated with neutrophilia.

'Aneosinopenia' may be defined as a condition in which there is a persistence of eosinophiles in the presence of a neutrophilic leucocytosis. Forty-one cases or 95 per cent of the cases showing neutrophilic leucocytosis were associated with aneosinopenia.

The asgmentopenia and aneosinopenia may be said to go almost hand in hand. One may state, therefore, that the presence of neutrophilic leucocytosis

with asegmentopenia and aneosinopenia is a feature which is not uncommon in malignancy, and which has been found to be of definite value in our effort to confirm a diagnosis of malignancy by hematologic criteria. It would not be justifiable, however, to assume that all carcinomas are associated with leucocytosis, for some cases (21 per cent in this series) present a definite leucopenia. It would be just as unjustifiable to assume that all cases present neutrophilia, for as will be pointed out, it is not unusual to find definite neutropenias (Table II).

Leucopenia—As was stated above 21 per cent of the cases presented a leucopenia below 8000. Five cases or 23 per cent presented a relative lymphocytosis. Furthermore, 10 cases or 47 per cent with leucopenia presented neutrophilia, 11 or 53 per cent presented neutropenia. Metastases were present in 12 instances or 57 per cent of the leucopenias (Table III).

Morphologic Changes in White Cells—These were studied in the ordinary smear stained by the Wright method.

Band Forms—Asegmentopenia. For purposes of this study, the normal upper limit of band forms in the blood was considered 5 per cent. Asegmentopenia was present in 85 per cent of the cases.

Asegmentophilia (Increase in band forms). This condition was present in 17 cases. When present, it was usually associated with either an irritative, infiltrative or destructive type of blood picture (see below). Simonis³¹ has called attention to the hypersegmentation of the neutrophiles, lymphocytes and monocytes, in the blood picture in carcinoma. His study, however, is based on only 11 cases.

Monocytosis—When the monocyte count was found to be 6 per cent or over, it was considered a monocytosis. Monocytosis was present in 28 cases. In 12 instances metastases were demonstrated.

Basophilia—Basophilia was absent in all but two cases of this series. There did not seem to be any significant relationship between this condition and metastasis.

Eosinophilia—Eosinophilia was present in 18 cases. In 7 instances it was associated with metastasis.

Metamyelocytes—These were present in small numbers in 7 cases.

Myelocytosis—This condition was present in 14 cases. The latter were associated with metastasis in 7 instances.

Osteoplasia and Osteoclasia—In 1891 Von Recklinghausen³² was the first to champion the conception of the spread of metastasis by the blood stream into the bone marrow. He differentiated two forms of bone marrow carcinoma. A. The osteoclastic form and B, the osteoplastic form. The osteoclastic form, by a process of resorption resembling osteomalacia causes brittleness of bone and may be accompanied by pathologic fractures (Table IV). But only extensive tumor invasion is capable of producing a marrow disturbance. In the osteoplastic variety hypertrophy of osteoid tissue with fibrotic changes occurs in the bone marrow and may proceed to calcification and sclerosis.

Slight osteoplasia which may be discernible by x-ray, was most commonly associated with blood pictures of irritative type. Moderate osteoplasia, which

TABLE II

LEUCOCYTOSIS INCIDENCE OF NEUTROPHILIA, ASEGMENTOPENIA AND ANEOSINOPENIA

ORGAN	NO OF CASES	TOTAL OF LEUCOCYTOSIS CASES	TOTAL WITH NO LEUCOCYTOSIS	LEUCOCYTOSIS	ASEGMENTOPENIA	ASEGMENTOPHILIA	NEUTROPHILIA PER CENT	NEUTROPENIA PER CENT	ANEO SINOPENIA
Breast	8	3	5	14,000 36,800 13,000	1 1 1	0 0 0	89 80	48	3 0 2
Lympho sarcoma	7	7	0	12,500 12,800 11,000 17,400 22,000 19,000 25,000	1 0 1 1 1 1 0	0 1 0 0 0 0 1	80 88 76 85 80	36	0 1 2 1 0 2 1
Sarcoma	5	1	4	10,000	1	0	82		1
Pancreas	6	4	2	18,400 10,700 11,000 11,000	1 1 1 1	0 0 0 0	 79	69 60 73	2 0 5 2
Gall bladder	4	4	0	17,200 15,000 19,200 16,000	1 1 1 0	0 0 0 1	86 88 86 81		2 1 1 1
Ovary	3	2	1	13,000 10,000	0 0	1 1	91	28	0 1
Urinary	5	4	1	12,200 9,600 11,500 13,800	1 1 1 0	0 0 0 1	75 89 77	62	2 5 0 0
Stomach	22	11	11	16,600 17,000 10,000 15,000 10,000 12,100 9,400 14,500 11,000 12,700 11,000	1 1 1 1 0 1 1 1 1 1 1	0 0 0 0 1 0 0 0 0 0 0	80 88 78 72 80 85 90 70	67 68 60	1 0 0.5 0 0.5 0.5 8.0 3.0
Colon	15	11	4	11,000 12,600 14,000 13,800 16,200 12,000 10,000 17,000 13,400 20,600 9,200	1 1 1 1 0 1 1 1 1 1 1	0 0 0 0 1 0 0 0 0 0 0	73 89 83 80 74 75 86 71.5 83	68 39.5	0 0 0 0 0 3 2 3 0 9.5 1

TABLE II CONT'D

ORGAN	NO OF CASES	TOTAL OF LEUCOCYTOSIS CASES	TOTAL WITH NO LEUCOCYTOSIS	LEUCOCYTOSIS	ASEGMENTOPENIA	ASEGMENTOPHILIA	NEUTROPHILIA PER CENT	NEUTROPENIA PER CENT	ANEOSINOPENIA
Lung	15	10	5	10,200	0	1			0
				14,000	1	0	78	69	0
				13,000	1	0	75		0.5
				12,000	1	0	75		3
				10,200	1	0	81		2
				15,000	1	0	77.5		1.5
				9,500	1	0		67	2
				12,000	1	0		56	7
				14,000	1	0		53	2
				10,900	1	0	79		0
Carcinoma of tongue	2	1	1	18,600	1	0	90		0
Metastatic carcinoma of bones (Primary not diagnosed)	3	1	2	36,800	1	0	89		0
Epithelioma of lip	1	1	0	12,000	1	0	89		0
Carcinoma of thyroid	1	1	0	13,000	1	0		67	1
Totals					52	9	43	18	41

may also be discernible on the x-ray film, was most commonly associated with blood pictures of an infiltrative type. Marked osteoplasia which is detectable by x-ray, was most commonly associated with blood pictures of a destructive type.

TYPES OF BLOOD PICTURES IN MALIGNANCY (TABLE V)

It was found that the blood pictures presented in malignancy could be classified into five groups: (1) Stimulative, (2) irritative or leucemoid, (3) infiltrative or dysplastic, (4) destructive or myelophthisic, (5) indeterminate.

Group I—Stimulative—This picture consists mainly of a slight leucocytosis (10-12,000), a moderate neutrophilia (75 to 80 per cent), the presence of asegmentopenia and no significant changes in the myeloid picture. If there are any changes in the lymphoid elements, there is usually an increase in the large variety. Erythrocytic changes are insignificant. This picture reflects a physiologic change in the bone marrow which is mainly one of hyperplasia. Leucocytosis and neutrophilia run parallel. Aneosinopenia is usually present.

This picture may be caused by the neoplastic toxin produced in the primary or metastatic focus. Case 71, carcinoma of the lung of a month's duration, is an example of this type. The blood picture was as follows: Hemoglobin 70 per cent, color index 0.83, red blood cells 4,260,000, white blood cells 10,200, polymorphonuclears 81 (Bands 0), Lymphocytes 16, Monocytes 1, Eosinophiles 2. One month later the smear revealed the following: Polynuclears 59 (Bands 0), Lymphocytes 26, Monocytes 3, Eosinophiles 12. This was the beginning of an infiltrative type of blood picture with perhaps slight osteoplastic changes, as

evidenced by the neutropenia and the increasing eosinophilia. The latter picture is probably the result of hyperplastic efforts on the part of neo-hematopoietic islands in the bone marrow—a compensatory effort, as it were, to replace infiltrated or compromised myeloid areas.

TABLE III
CHART OF LEUCOENIAS

ORGAN	NO. OF CASES LEUCOPENIA	LEUCOPENIA	NEUTROPENIA OR NEUTROPHILIA	LYMPHOCYTOSIS OR LYMPHO- CYTOPENIA	METASTASES
Breast	1	7000	78	18	+
Spleen	2	3800 6000	54 59	47 33.5	+
Pancreas	2	6300 7500	67 75	21 15	0 0
Ovary	1	6200	72.5	9	+
Uterus	1	7400	84	14	+
Stomach	5	2400 6800 7800 5700 5800	88 62 60 92 70	8 32 28 16 26	+
Colon	2	6600 7500	64 73	33 25	0 0
Lung	2	7650 2400	71 24	25 56	+
G. I. Malignancy	2	3600 4200	58 70	38 20	+
Carcinoma of Tongue	1	7400	69	28	+
Metastatic Carcinoma of Bones	1	6700	58	32	+
Carcinoma of Tonsil	1	6600	57	36	+
Total	21				12

TABLE IV
OSTEOPLASIA AND TYPES OF BLOOD PICTURES

DEGREE	BLOOD PICTURE GROUP
Slight	Irritative
Moderate	Infiltrative
Marked	Destructive

Note—Bone Marrow carcinoma may be productive of osteoplastic and osteoclastic changes, either of which may predominate, to the complete or partial exclusion of the other.

TABLE V
TYPES OF BLOOD PICTURES IN MALIGNANCY

STAGE	TYPE OF PICTURE	LEUCOCYTOSIS	NEUTROPHILIA	LEUCOPENIA	RELATIVE LYMPHO CYTOSIS	ASGMFN TO ENLA	ASGMFN TO PHILIA	ANEO SIND PENIA	MYELOID CHANGES	LYMPHOID CHANGES	ERYTHRO CYTIC CHANGES	BONE MARROW TAPHY	REMARKS
I	stimulative hypertrophic	slight	slight			present		usually present	insignificant	increase in large lymphatic type	insignificant	hyperplasia rapid decrease of cells	leucocytes and neutrophils run parallel
II	irritative hyperplastic leucemoid osteoplasia slight	moderate		may be present		may be present	may be present	usually present	occasional myelocytes or metamyelocytes		shift to left, slight mononuclear increase in reticulocytes	hyperplasia increase in cell growth	leucocytosis and neutrophilia may be disproportionate
III	infiltrative dysplastic osteoplasia moderate		slight	slight	slight	usually absent but may be present	present or absent	not important	myelocytes and metamyelocytes present	relative lymphocytosis, increase in large lymphs	shift to left moderate	microscopic metastases of moderate osteoplasia	
IV	destructive myelophthasic osteoplasia marked				moderate	usually absent	usually present	not important	myelocytes metamyelocytes and myeloblasts present		shift to left	microscopic metastases of marked osteoplasia	
V	metastatic			absent		absent					insignificant		

Group 2—Irritative, Leucemoid Slight Osteoplasia—This picture consists of essentially a moderate leucocytosis (12,000 or more) and a moderate neutrophilia (80 per cent or over). Asegmentophilia is usually present and assegmentopenia may sometimes occur. Aneosinopenia is usually present. There may be an occasional myelocyte or metamyelocyte present and there are no changes in the lymphoid elements. Erythrocytic changes may be manifested in the presence of increased reticulation or anochromasia. The bone marrow pathology is essentially one of hyperplasia. In this group, neutrophilia may be disproportionate to leucocytosis and indeed even neutropenia may occur. Case 74 a carcinoma of the hepatic duct, whose history dates back 18 months, is an example of the irritative type of blood picture in malignancy. Hemoglobin 20 per cent, color index 0.32, red blood cells 3,100,000, white blood cells 16,000 polynuclears 81 (Bands 15), lymphocytes 10, monocytes 8, eosinophiles 1. There were no particular changes in the red cells. This picture differs from the stimulative form by a shift of the neutrophils to the left. In spite of this eosinophiles are found (aneosinopenia). This picture may be the result of microscopic metastasis in the red marrow areas. Such metastasis may be detectable on the x-ray film. On the other hand slight osteoplastic changes may suffice to produce the same result.

Group 3—Infiltrative, Dysplastic or Moderate Osteoplasia—Essentially, the blood picture consists of a leucopenia (5 to 8000), a neutropenia (50 to 60 per cent) and a relative lymphocytosis (30 to 40 per cent). If neutrophilia is present, there is usually a combination with Group 2. Asegmentopenia is usually absent but may be present. Asegmentophilia is usually present but may be absent. There may be present myelocytes and metamyelocytes. Should there be a relative lymphocytosis, there is a shift to the left that is, the large lymphocytes are increased. Erythrocytes usually show moderate changes as manifested by hypochromasia, anochromasia, polychromasia, and occasional normoblasts. Pathologically, the bone marrow may be the seat of microscopic metastases or a moderate degree of osteoplasia. The metastases may or may not be discernible by x-ray. Typical of this type of picture is the one observed in Case 78, with a carcinoma of the uterus and a history dating back three months. Here the blood study presented the following: Bleeding time one minute forty-one seconds, coagulation time, seven minutes ten seconds, tourniquet test negative, no significant changes in the fragility of red cells, hemoglobin 42 per cent, color index 0.89, red blood cells 2,400,000, platelets 190,000, white blood cells 8600, polynuclears 67 (Bands 0), lymphocytes 25, monocytes 3, eosinophiles 3, myelocytes 2, slight anisocytosis, slight macrocytosis, moderate anochromasia, moderate hypochromasia, and an occasional normoblast.

The leucocyte count tends toward a leucopenia. Neutrophilia is declining toward a neutropenia. In other words, there is evidence of beginning bone marrow dysfunction, and at the same time, there is evidence of irritative phenomena. The latter is manifested by the presence of occasional normoblasts, myelocytes and metamyelocytes. Although there is displacement of myeloid tissue by the metastatic focus, or possibly by the presence of moderate osteoplasia, there is still enough myeloid tissue left which is capable of being irritated and responding to a stimulus.

Group 1—Destructive, Myelophthisic or Marked Osteoplasia—Essentially, the blood findings in this group are as follows: a moderate leucopenia (below 5000), a moderate neutropenia (below 50), a moderate relative lymphocytosis (over 40), asegmentopenia is usually absent, asegmentophilia is usually present, myelocytes, metamyelocytes, and myeloblasts may be present. If relative lymphocytosis is present, there is a shift of the lymphocytes to the left. Erythrocytic changes may be manifested by the presence of normoblasts, reticulocytosis, and polychromasia. Pathologically, there may be macroscopic metastasis in the bone marrow or the presence of a moderate degree of osteoplasia. These are revealed on x-ray.

The picture aforementioned is probably produced by the neoplasm in its progressive invasion of myeloid tissue to its ultimate destruction or displacement, or by the presence of moderate or extensive osteoplasia. Case 98, a carcinoma of the lung with a history dating back two weeks, is a typical example of such a type. He presented the following blood picture: Hemoglobin 85 per cent, color index 0.88, red blood cells 4,800,000, platelets 250,000, white blood cells 2400, neutrophils 24 (Bands 0), lymphocytes 56, monocytes 11, eosinophiles 1, metamyelocytes 4, myelocytes 1, and 5 normoblasts.

The above picture represents a combination of leucopenia, neutropenia, relative lymphocytosis with a shift of the neutrophils to the left (due to metamyelocytosis). One would expect in this type of case, a diminution in platelets because of this neoplastic panmyelophthisis, but whether the megakaryocytic foci are relatively uninjured or the accessory reticuloendothelium is responsible for compensatory regeneration, one can only surmise. Further observations along this line are necessary to clarify this point. This patient also showed a purpura in spite of the normal platelet count. It may be possible that platelet function was impaired.

These groups are not completely delimited and may overlap depending upon character and extent of the metastatic involvement or osteoplasia. Case 71, for example, showed two types of pictures during its course. The first picture was a stimulating one, the second of an irritative type.

Changes of type of blood picture may be induced by treatment, including x-ray or radium by operative intervention, or by the mere passage of time. Case 17, for example, with a carcinoma of the stomach, showed a preoperative picture which was definitely infiltrative. After operation, the picture was definitely irritative. Case 20 with metastasis from carcinoma of the stomach, presented a picture of beginning infiltration three months previous to admission to the hospital. The picture changed to that of definite infiltration with irritative marrow changes on admission to the hospital three months later. Case 37, with sarcoma of right humerus, presented on admission a blood picture of the destructive type. This was due to x-ray therapy received one month previous. A month later, he showed a similar picture and in addition a thrombocytopenia of 60,000 and signs of bone marrow irritation, as evidenced by definitely increased reticulocytosis (3 per cent). Case 71 entered the hospital with a diagnosis of carcinoma of the stomach. He presented a picture of the infiltrative type. One month later, there was increasing neutropenia and relative lymphocytosis with evidence of erythrocytic and myelocytic irritation, the picture thus becoming

definitely irritative in type. Case 91, with carcinoma of the head of the pancreas, entered the hospital with an indeterminate type of blood picture. One month later there was a typical stimulative type of blood picture thus indicating the influence of the time factor alone. Case 94, with carcinoma of the colon, was admitted with a stimulative blood picture. One month later the picture became definitely infiltrative. Case 97, with a carcinoma of the sigmoid, was admitted with a picture of the stimulative type. Two weeks later there were beginning signs of an infiltrative type of blood picture.

Group 5—Indeterminate—This group consists of those cases in which the criteria outlined above are indefinite or insufficient for accurate interpretation. The question of time alone, the influence of treatment or operative intervention may suffice to initiate a picture which later becomes definite. These cases ultimately pass into the infiltrative or destructive groups. Case 17, for example, a case of carcinoma of the stomach was admitted on the medical service with an indeterminate blood picture with the following differential count: polynuclears 47 (Bands 1), lymphocytes 34, eosinophiles 2, monocytes 8, and slight oligochromasia. He was transferred to the surgical service for exploratory laparotomy. Two days after the operation he presented the following blood picture: bleeding time twenty seconds, coagulation time seven minutes twenty seconds, hemoglobin 80 per cent, color index 0.7, red blood cells 5,600,000, platelets 350,000, WBC 10,000, polynuclears 80 (Bands 39), metamyelocytes 4, myelocytes 2, and slight anochromasia. This was a blood picture quite typical of the early infiltrative type. It is possible that in this case there was esophageal involvement also, as suggested by the high hemoglobin and red cell count.

Incidence of Types of Blood Pictures in Malignancy (Table VI)—Group I—or stimulative group, was present in 55 cases or 55 per cent of this series.

Group II—or irritative group, was present in 16 cases or 16 per cent of this series.

Group III—or infiltrative group, was present in 9 cases or 9 per cent of this series.

Group IV—or destructive group, was present in 7 cases or 7 per cent of this series.

Group V—or indeterminate group, was present in 13 cases or 13 per cent of this series.

It is obvious then, that at the time of the blood examination, over half of the cases were in the stimulative group, one sixth in the irritative group, one-eleventh in the infiltrative group, one-fourteenth in the destructive group, and one-eighth in the indeterminate group.

It is interesting to enquire into the feasibility of utilizing the blood study early enough to be of benefit to the patient. One must conclude that at this time it is practicable only in the stimulative group before the onset of metastasis. For purposes of ultimate prognosis and treatment, the stimulative group may be divided into two subgroups, (a) without metastasis, (b) with metastasis.

Table VII, presents an analysis of these subgroups. It is found that of 55 cases which present a stimulative blood picture, 32 or 60 per cent have no demonstrable metastasis. Theoretically, it should be possible then to diagnose

TABLE VI
ANALYSIS OF BLOOD PICTURE IN MALIGNANCY

ORGAN	TOTAL	STIMULATIVE NO OF CASES	IRRITATIVE NO OF CASES	INFILTRATIVE NO OF CASES	DESTRUCTIVE NO OF CASES	INDETER- MINATE NO OF CASES
Breast	8	5	2	1		0
Lympho- sarcoma	7	7		0		
Sarcoma tosis	5	1	1		1	2
Stomach	22	9	3	4	3	3
Lung	15	8	3	1	1	2
Colon	15	1	1			2
Pancreas	6	4	1			1
Gall bladder	4	3	1			
Ovary	3	2	0	1		
Urinary	5	2	2	1	0	0
Carcinoma of tongue	2	1				1
Epithelioma of lip	1	1	0			
Carcinoma of tonsil	1				1	
Carcinoma of thyroid	1	1				
Metastatic carcinoma of bones	3	0	2	0	1	
Uterus	2					2
Total	100	55	16	9	7	13

malignancy hematologically in almost one-third of the cases at a time when metastasis is clinically absent. The establishment of this possibility as a fact opens up a field which needs further study.

GENERAL METASTASIS AND THE BLOOD PICTURE

Forty-nine cases, presented metastasis. Table VIII, presents an analysis of the incidence of the five groups of blood pictures that present themselves in cases with metastasis. Metastasis may affect a blood picture in two ways: (1) by the specific effect of malignant substance on the bone marrow and (2) by the mechanical presence of metastatic tissue in the bone marrow. There may be a combination of both factors. In the first instance the picture produced may be either stimulative or irritative, in the second instance, the resulting blood picture may be irritative, infiltrative, or destructive in type. It is found that 40 per cent of cases with metastasis in this series were in the stimulative group, 24 per cent in the irritative group, and 8 per cent each in the destructive and indeterminate groups.

BONE MARROW METASTASIS AND THE BLOOD PICTURE (TABLE IX)

Twelve cases, or 12 per cent of the entire series, presented evidence of metastasis to the bone marrow. In 9 cases there was x-ray evidence and in 3 instances autopsy evidence. Fifty-eight per cent of cases with bone marrow

metastasis showed an irritative type of blood picture Twenty-five per cent showed an infiltrative blood picture Sixteen had a blood picture of the destructive type It is significant that there were no stimulative or indeterminate types of picture in this group

TABLE VII
ANALYSIS OF THE STIMULATIVE GROUP OF BLOOD PICTURES

ORGAN	TOTAL NO OF CASES	TOTAL STIMULATIVE	WITH METASTASIS	WITHOUT METASTASIS
Breast	8	7	3	2
Lymphosarcoma	7	7	1	3
Sarcoma	5	1	0	1
Stomach	22	9	2	7
Lung	15	8	0	2
Colon	15	12	3	9
Pancreas	6	4	1	3
Gall bladder	4	3	2	1
Ovary	3	2	1	1
Urinary	5	2	1	1
Epithelioma of lip	1	1	0	1
Carcinoma of thyroid	1	1	0	1

TABLE VIII
ANALYSIS OF BLOOD PICTURE IN METASTATIC MALIGNANCY

ORGAN	METASTASIS NO OF CASES	STIMULATIVE NO OF CASES	IRRITATIVE NO OF CASES	INFILTRATIVE NO OF CASES	DESTRUCTIVE NO OF CASES	INDETER- MINATE NO OF CASES
Breast	6	3	2	1	0	0
Lympho- sarcoma	4	4				
Sarcoma	2	0	1		1	
Stomach	10	2	2	1	3	2
Lung	11	6	3		1	1
Colon	2	2				
Pancreas	1	1				
Gall bladder	3	2	1			
Ovary	2	1	0	1		
Prostate	1		0	1	0	
Hyperneph- roma of kidney	1	1				
Adenocarci- noma of kidney	1	0	1			
Tongue	1					
Carcinoma of tongue	1					
Metastatic carcinoma of bones	3	0	2	0	1	

The irritative type of blood picture produced by bone marrow metastasis may be accounted for by hyperplasia of the bone marrow with or without the association of *slight* osteoplastic changes The infiltrative type of blood picture produced by bone marrow neoplasia may be due to the early invasiveness of the tumor with or without *moderate* osteoplastic changes The destructive type of

TABLE IX
ANALYSIS OF BONE MARROW METASTASES AND TYPES OF BLOOD PICTURES

CASE NO	ORGAN	DIAGNOSED BY	STIMULATIVE	IRRITATIVE	INFILTRATIVE	DESTRUCTIVE	INDETERMINATE
2	Ovary	X Ray			+		
11	Tonsil	X Ray				+	
32	Bones	X Ray		+			
35	Stomach	Autopsy		+			
39	Lung	X Ray		+			
40	Lung	X Ray			+		
43	Breast	X Ray		+			
45	Stomach	X Ray		+			
60	Lung	X Ray		+			
83	Breast	Autopsy			+		
87	Adenocarcinoma of kidney						
98	Lung	X Ray Autopsy		+		+	

picture produced by bone marrow metastasis, may be accounted for by the invasion of a great extent of the marrow by the advancing neoplasm with or without *marked* osteoplasia

Von Recklinghausen¹³ accepted the mechanical interpretation of the effect of metastasis on the marrow. He believed that there was brought about obstruction to the venules and later larger vessels causing a hypostatic congestion and active congestion of the bone marrow, which in turn led to stimulation of the marrow elements.

Schmoil and Oxhausen¹⁴ adopted a biologic interpretation believing that the changes brought about in the marrow were mainly due to the specific or biologic reactivity of the marrow. Zadek and Sonnenfeld¹⁵ believe that both mechanical and biologic elements must be factors in an explanation of the reactivity of the bone marrow in carcinoma of the bone marrow. In the study of three cases of prostatic carcinoma they concluded that they could differentiate hematologically between the two groups of marrow carcinoma. They summarized their findings in osteoplastic carcinoma as follows: Secondary anemia, leucopenia, relative lymphocytosis, occasional normoblast, the picture on the x-ray film was characteristic. The osteoclastic blood picture they described as follows: normoblastemia, leucocytosis, with varying grades of nuclear shift and relative lymphocytopenia.

It can readily be seen in the light of this study that their conclusions were unwarranted, because as has been shown four definite groups based on the blood picture can be distinguished depending either on the effect of the malignant substance from the primary or metastatic focus upon the bone marrow or on the mechanical effect of the presence of an infiltrating mass compromising and destroying bone marrow substance. The picture described by Zadek and Sonnenfeld as due only to marked osteoplastic changes could just as well be produced by changes other than those predominantly osteoplastic. Similar blood pictures as already pointed out can be produced by any lesion which exerts an infiltrative or destructive effect on the marrow. Weber and Bodt¹⁶

report a case of prostatic carcinoma with hyperchromic anemia, leucocytosis, marked normoblastemia, myelocytosis, in which autopsy revealed marked osteoplastic changes, thus again negating the belief of Zadek and Sonnentfeld⁹ that osteoplastic bone marrow metastasis can be diagnosed hematologically by leucopenia, relative lymphocytosis with occasional normoblast

COMMENT

The diagnosis of malignancy may be suspected or corroborated hematologically, not by any one particular finding, but by a combination of findings, a constellation of hematologic facts as it were. The bleeding time is only very rarely prolonged, usually less than 1 minute. The coagulation time is within normal limits. The platelets are normal or increased. An increase explains the frequent occurrence of nonmalignant thrombosis in malignancy. The tourniquet test is rarely positive. The fragility test shows no significant changes.

Leucocytosis is present in approximately two thirds of the cases and is associated with assegmentopenia. In 85 per cent of the cases presenting leucocytosis 43 per cent of these are associated with neutrophilia. Of the latter, 95 per cent are associated with an aneosinopenia. Assegmentopenia and aneosinopenia, therefore, go hand in hand.

The red cells show a slightly depressed count in two-thirds of the cases a markedly depressed count in one-eighth and normal or increased values in one-fifth. There is never a count of less than one million in the absence of hemorrhage. Changes in red cell morphology are conspicuous by their absence, the most frequent finding being anochromasia (52 per cent of the cases). The least frequent changes are macrocytosis, normoblastemia and polychromasia.

No matter how complete the evidence of malignancy may be on hematologic grounds, a most important factor in diagnosis still remains that is the clinical picture of the patient as determined by the history and physical examination of the patient. Experience teaches that the blood picture of malignancy may be simulated in other conditions such as Hodgkin's disease, severe diabetes and diabetic coma, severe nephritis or incipient uremia, cardiac decompensation and cardiac asthma.

A practical point worthy of note is the fact that a leucopenia is associated with metastasis in over half the cases. Thus given a case of malignancy where operative intervention is being considered a leucopenia favors metastasis and the patient may therefore, be spared unnecessary surgical intervention, or the burden of x ray or radium therapy.

SUMMARY

1 One hundred proved cases of malignancy were studied hematologically and were viewed from a clinicohematologic aspect.

2 Blood pictures were classified into four distinct groups and one indeterminate group according to definite hematologic constellations.

3 Metastasis in bone marrow was discussed from an anatomic, pathologic, and hematologic viewpoint.

4 Hematologic data may be used to diagnose cases presenting stimulative

nonmetastatic blood pictures. This is possible in one-third of all the cases of malignancy. More frequent studies may even lead to earlier recognition of malignancy and consequently open up opportunities for greater therapeutic possibilities.

5 Presence of metastasis may be diagnosed long before clinical manifestations are present and may spare the patient operative risk or unnecessary treatment.

6 Unexplained leucocytosis may be ascribed to malignancy and the latter considered, especially if associated with assegmentopenia and aneosinopenia.

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THE ACID RESPONSE OF THE STOMACH TO TEST MEALS OF PROTEIN, FAT, AND CARBOHYDRATE*

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THE reaction of various parts of the gastrointestinal tract to different foods has been a fertile field for investigation for many years. In spite of this our conceptions of the biochemistry involved are vague and uncertain, being too often based on preconceived ideas or on conclusions drawn from results obtained from incorrect methods or on experimental work which has disregarded certain variables.

Rehfuss, Hawk and Berghem¹ carried on, over several years, an exhaustive investigation into the emptying time of the stomach and the acid response of the stomach to all types and varieties of foods. The chart shown herewith, Chart I which is based on the report of these findings, indicates that the emptying time of the stomach with a given food varies as the acidity of the gastric contents with that same food.

Hochzel,² who did 2 000 aspirations on himself, studied the effect of fasting on the gastric acidity and followed this by a study of gastric reaction to meat, egg and vegetable protein. He concluded that these products following a period of fasting, lowered the acidity of the gastric contents.

The difficulty in drawing exact conclusions from much of the work that has been done lies in the fact that we are in each case dealing with a mixture of unknown proportions of food ingested and gastric juice and that in no case was any allowance made for the buffer value of the test meal. Thus although one can say that the P_H of the aspirated contents varies with certain foods, it is a fallacy to speak of the acid response of the stomach without taking these factors into consideration. Graham and Emery³ studied the permanent or prolonged reaction of various parts of the intestinal tract of the dog to four different diets. These dogs were fed one of the following diets over a period of several weeks: normal diet of meat and bread, a protein diet of meat only, a carbohydrate diet of bread only, and a fat diet consisting of lard with an allowance of bread to prevent acidosis. These dogs were killed twenty-four hours after a feeding in order to reduce to a minimum the chemical reaction in the food itself, and the gut was immediately tied off at different levels. The contents of each section were then examined for their hydrogen-ion concentration. The contents were found to be less acid the further they were from the pylorus, but the difference in P_H was in no instance greater than 1.7. There was no evidence that the variation in diet influenced the P_H of the tract secretion.

Essentially the same results were reported by Grayzell and Miller⁴ except that some of their dogs were fed a rachitic diet and these animals responded with a somewhat less acid reaction of the intestinal secretion.

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Dean¹ examined the contents of the terminal ileum on dogs with fistulas and on a dog with an anastomosis of the ileum to the anus. After a high fat meal the average P_{H} was 7.42, after a high protein meal 7.86, after a high carbohydrate meal 7.42, and after a diet of whole milk 7.86. Although not in accord with other investigators who found the intestinal contents of the dog to be uniformly acid, it is worthy of note that again in this investigation there is no evidence that the kind of food eaten radically affects the P_{H} of the contents of the intestinal tract.

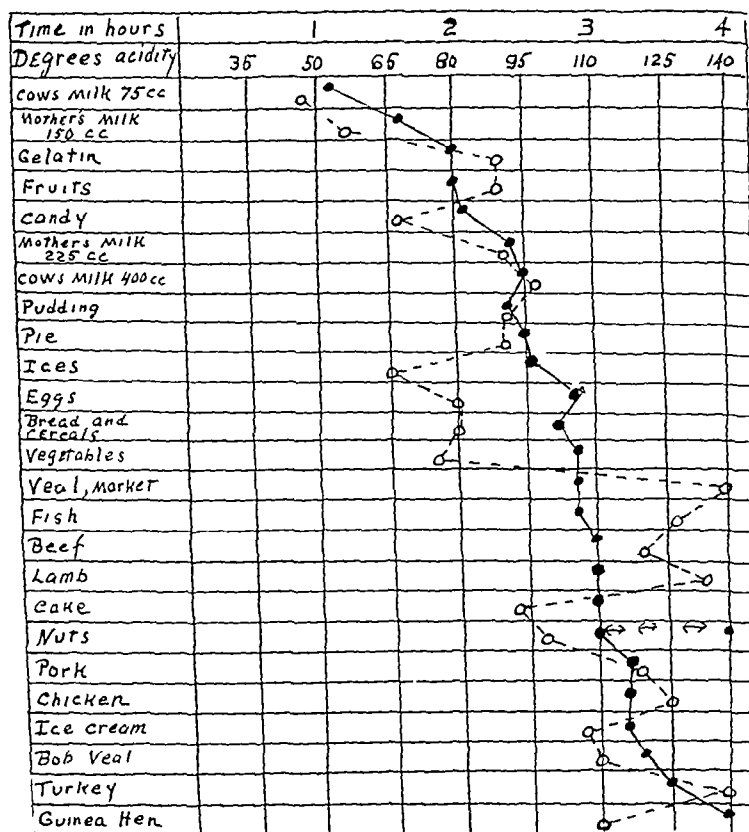


Chart I—Emptying time of stomach (dark circles and solid line) and acidity of gastric contents (light circles and dotted line) with various test meals. From figures reported by Rehfuess, Hawk, and Bergheim.

Recently Bloomfield and his collaborators⁶ have standardized the determination of the acid content of the gastric juice in response to an alcohol meal and to histamine given hypodermically. By the use of phenolphthalein as an indicator in the meal it is possible to estimate exactly the dilution of the aspirated contents by the meal and consequently to determine the acidity of the pure gastric juice. Furthermore the negative buffer value of the alcohol meal eliminates a source of error never taken into consideration in the old Ewald test meal or similar methods. With accurate determination of the reaction of the stomach through its acid secreting apparatus thus made possible an effort has

been made to determine the response of the stomach to the various food elements, namely, fat, protein, and carbohydrate. Certain difficulties presented themselves which have resulted in my being able to report on only a relatively small series. Subjects with essentially normal gastrointestinal tracts who would submit to repeated gastric analyses were not easy to find, and many of the analyses made had to be discarded because of persistent regurgitation of bile in the course of the tests, indicative of a consequent dilution of the gastric contents with intestinal secretion. Nevertheless there are reported herewith the results of the gastric analyses on twenty subjects using the test meals mentioned above.

The procedure followed was a modification of the Bloomfield method of gastric analysis with the alcohol test meal which was used as a comparative standard. A Rehfuess tube was inserted and the fasting contents aspirated. With the tube still in place the test meal was introduced in the stomach through the tube by the use of a large syringe. At fifteen minute intervals for one hour the whole gastric contents were aspirated, the amount noted, a sample saved for examination and the remainder returned to the stomach. A determination was made on each sample of the free HCl and total acid estimated in degrees of acidity with diamethylamino azobenzol and phenolphthalein as indicators. The protein meal was 50 cc. of a 5 per cent (by weight) suspension of dry egg albumin in water. Determinations of the proportion of the meal to pure gastric juice in the aspirated contents were based on colorimetric readings with phenolphthalein of known concentration in the meal and also by Esbach determinations on the fasting contents, on the test meal, and on the gastric contents at intervals after the introduction of the meal.

The fat meal consisted of 50 cc. of pure olive oil. Some difficulty was encountered in completely emptying the stomach at each 15-minute interval because of the viscosity of the oil, but since my observations were chiefly concerned with the acid concentration of the gastric secretion rather than with the amount and since the aspirated contents were returned to the stomach except for the sample saved for analysis, this point is of little significance. Separation of the fat from the gastric secretion was accomplished by the use of caprylic acid to break up the emulsion and thus the proportion of meal to gastric secretion was determined by direct reading. As before this correction was used in the titration of the HCl and total acidity of the aspirated contents, so that the reported figures indicate the acidity of the pure gastric juice.

The carbohydrate meal was 50 cc. of a 5 per cent glucose solution. Phenolphthalein in solution (0.5 cc. of a 1 per cent solution) was added to the glucose solution for the colorimetric determination of the dilution of the gastric secretion by the meal. Sugar determinations were also made in several instances as a check on these readings.

One of the subjects on whom the gastric analyses mentioned above were being run was given, by mistake, a drink of pineapple juice several hours before the test meal. Since this was not discovered until after the tube had been passed, and thinking that it might be of interest to see whether or not it affected the acid response of the stomach, the gastric contents were aspirated in the usual fashion and thrown away, and the patient was then given a test meal of

olive oil This subject had shown an achlorhydria in response to meals of glucose, egg albumin, and alcohol, although with histamine he had a hydrochloric acid response that was about normal It was surprising to note that in response to the meal of olive oil given after the ingestion of the pineapple juice, the hydrochloric acid readings in the samples aspirated were also about normal Because of these findings pineapple juice was added to the test meals on subjects examined from that time on Analyses of canned pineapple juice are submitted herewith, and it is to be supposed that any increased acid response in the stomach following the use of this fruit juice as a test meal, is due to the citric acid present

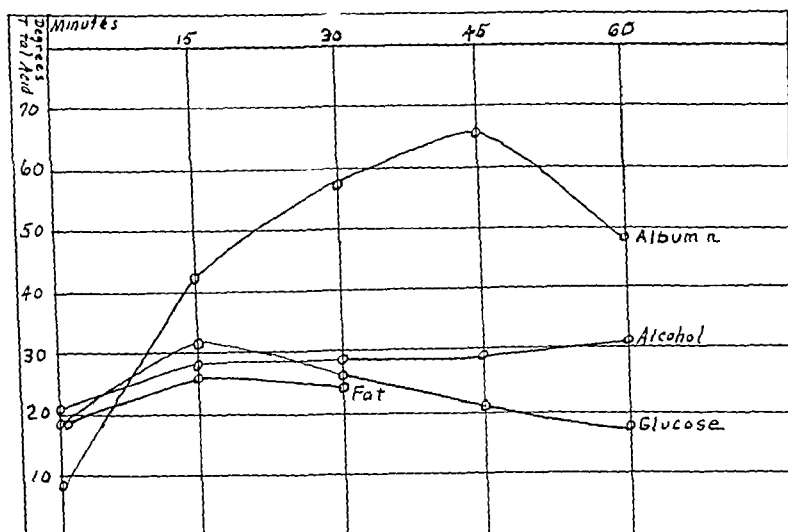


Chart II—Subject No 2 Acid response of the stomach in degrees of total acid in response to meals of albumin alcohol fat and glucose

Analysis	
Unsweetened Pineapple Juice	
(National Canners Laboratory—2/12/31)	
Total Solids (70° C in Vacuum)	12.39 per cent
Total Sugars—as invert	9.97
Total Acidity—as citric acid	1.17
Protein (N x 6.25)	3.4
Ether extract	5.6
Ash	4.0

In estimating the total acidity after a fruit juice test meal, allowance was, of course, made for the citric acid present in the meal so that as before the reading given indicates the total acidity of pure gastric juice Two charts are shown indicating in two subjects the response of the stomach to the various meals mentioned above The first subject (Chart II) received test meals of albumin alcohol glucose, and fat, and although the range of acidity was not great namely 40 degrees the acid response was as indicated namely, the greatest from albumin and the least from fat The other subject whose findings are charted herewith (Chart III) was the one who received the fruit juice in error The degree of acidity for the fat meal following the fruit juice could be read in

terms of HCl only the dilution with the acid fruit juice being an unknown quantity. These readings are represented by the dotted line. The solid line represents the estimated probable total acidity.

Chart IV gives the composite curves of all analyses on all subjects. In order to draw any definite conclusion from such a record each subject should have received the same test meals, but regurgitation of bile or the unwillingness of the patient to proceed with this series of tests, or his discharge from hospital, interfered with the complete course being run in each case. The number of tests included in each of the curves given differs. Nevertheless these analyses are all from the same group of subjects each subject had two or more test meals and the composite curves may, therefore be taken to be indicative of the general character of the curves of a larger and more complete set of analyses.

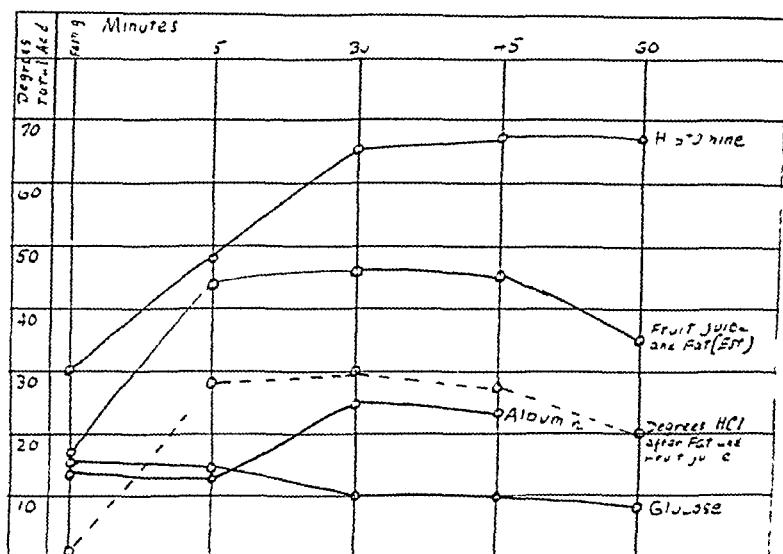


Chart III—Subject No. 6. Acid response of stomach to various test meals and to histamine. (See text.)

The point will possibly be raised that the acid response of the stomach might vary from day to day regardless of the type of meal used. It has been definitely shown, however,⁶ that the acid response of the stomach to a standard test meal is a very constant factor not only from day to day but over a long period of time.

It will be noted on this chart (Chart IV) that the greatest acid response of the stomach was obtained by the use of histamine. This of course is in accord with previous reports.⁶ Albumin injected into the stomach was the next most active, with pineapple juice, glucose, fat and alcohol coming as next most active in the order named. It will be noted, however, that the greatest difference in degrees of acidity with any of the test meals excluding alcohol was less than 30, and at the end of one hour when the last sample was taken was less than 15.

These findings would seem to be in accord with the reports of the acid response of the other parts of the intestinal tract referred to earlier, namely, that the character of the food ingested does not materially affect the acid re-

sponse of the stomach. It is possible that from the practical and clinical point of view the findings as charted might indicate that fat would be less apt to aggravate a hyperchlorhydria than would any other of the food elements introduced, and conversely that fruit juice and albumin might be indicated in hypochlorhydria. Sansum advocated the use of the citrus fruits in hypochlorhydria,⁷ but there is no record that there were any gastric analyses done to show that this therapeutic regime would in any way affect the acid response of the stomach. Moreover from the clinical point of view the question must be raised as to whether there are any subjective symptoms from hypochlorhydria or hyperchlorhydria per se, or whether, granting that there are such symptoms, any difference in the composition of the diet with respect to its albumin, fat, glucose, or acid content could be great enough to change the in-

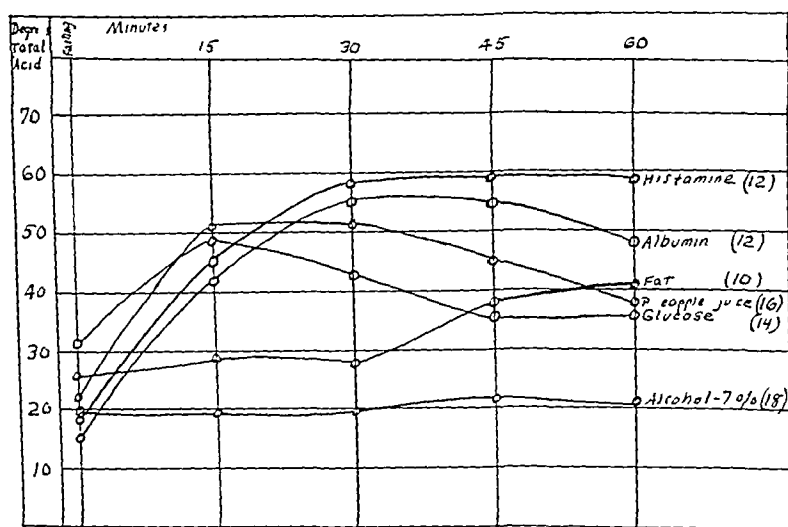


Chart IV—Composite curves showing acid response of stomach in response to various test meals and to histamine on all subjects. The total number of subjects studied was twenty. The number of test meals included in each curve is indicated by the figures at the right.

tensity of these symptoms. It would seem at least from the findings reported herewith that the physical composition of the food might be of more importance than its chemical composition.

SUMMARY

Previous investigations do not definitely indicate that there is any factor that will affect the acid response of the stomach other than the effect of various test meals on the emptying time of the stomach, the degree of acidity being greatest with foods which are retained longest in the stomach.

Experimental work on dogs shows that there is no evidence that variation in diet, or the kind of test meals given radically affects the pH of the secretion of the small intestines.

This report covers the results of gastric analyses obtained after test meals of protein, fat, and carbohydrate in the form of egg albumin, olive oil, glucose, and of fruit juice. The averages of the figures obtained from all analyses in-

dicates that there is a slight difference in the response of the stomach to these various food elements, but that the difference is not great enough to be of any theoretical significance, or practical importance. These findings are in accord with the reports of findings previously mentioned on the effect of diet on other parts of the intestinal tract.

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THE LEUCOCYTES IN SURGICAL CONDITIONS*

A STUDY OF 275 CASES

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THE valuable information in prognosis given by the Schilling¹ hemogram as compared to the older Ehrlich differential count is pointed out in recent contributions by Reznikoff,² Weiss,³ Boies,⁴ Alden,⁵ and DeMotte, Goodale⁶ and others. The earlier teaching was that a high total count with a low polymorphonuclear percentage carried a good prognosis, whereas a low total count with a high polymorphonuclear count indicated a more serious outlook. The Schilling hemogram method considers, in addition, the morphologic variations in the polymorphonuclears and of the two other great systems of leucocytes, the mononuclears and lymphocytes. Sabin⁷ has made clear the interrelationship of this floating supply of leucocytes to the bone marrow organ. The purpose of this study is to tabulate the findings in 275 surgical cases and attempt to correlate the leucocytic picture with the clinical condition.

The first series consisted of 213 cases studied by the Ehrlich differential count. The proportion of total count to polymorphonuclears is expressed by the Gibson⁸ index shown in Chart 1.

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Cases are grouped into three classes. The uninfected group includes hemorrhoids, hernias, chronic appendicitis, and pelvic repair operations. A second group of moderately infected cases consists of furuncles, superficial infections, incomplete abortions, acute catarrhal appendicitis and acute suppurative salpingitis. A third group is made up of acute gangrenous appendicitis.

TABLE I
SUMMARY OF 213 SURGICAL CASES STUDIED WITH TOTAL COUNT AND EHRLICH DIFFERENTIAL

DIAGNOSIS	NO. CASES	TOTAL WHITE COUNT				POLYMORPHONUCLEARS					GIBSON INDEX		
		UNDER 10,000	10-15,000	15-20,000	ABOVE 20,000	BELOW 60	60-70	70-80	80-90	90-100	PRO. PORTION ATF	DIS. PORTION ALE	PER CENT PORTION ATE
Clean Cases	112	68	37	7	-	21	61	26	4	-	104	8	90
Moderate Infections	72	14	24	25	9	1	16	37	16	2	62	10	72
Severe Infections	29	1	9	9	10	-	-	7	20	2	16	13	54

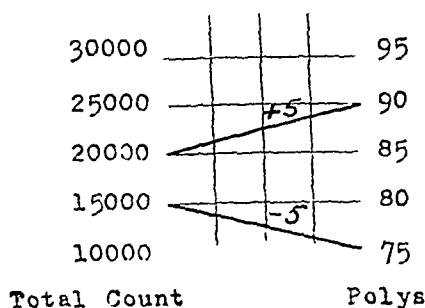


Chart 1.—Gibson Standard chart. Ten thousand white cells and 75 per cent polymorphonuclears are taken as the base line. Increase of one in the polymorphonuclear count should be accompanied by a proportional increase of one thousand in total count. An increase of polymorphonuclears out of proportion to the total count will cause the line depicting the count to slant upward. Each division above horizontal is called a plus unit. Minus units are produced by the line slanting downward to the right.

If one studies the distributions in Table I there is seen to be a definite tendency to increasing total count and increasing polymorphonuclear count as the seriousness of the infection increases. Also, with increasing seriousness of infection the normal proportion of total count to polymorphonuclears decreases from 90 per cent in the clean cases to 54 per cent in the acute gangrenous appendicitis.

In the Schilling counts 3 to 5 per cent of staff cells is considered normal. Rarely up to 1 per cent of juveniles may occur, making six immatures the upper limit of normal. In colds, superficial infections and gastro intestinal upsets an increase of one to two cells may or may not occur. In infections of moderate grade, 10 to 15 per cent immatures may be found. Severe or overwhelming infections may show 25 to 50 per cent.

TABLE II

SUMMARY OF 62 SURGICAL CASES STUDIED WITH SCHILLING HEMOGRAM AND GIBSON INDEX

	NO CASES	PROPORTIONATE		DISPRO- PORTIONATE	IMMATURES BELOW 6	IMMATURES ABOVE 6	PER CENT BELOW 6
Clean Cases	33	33	100%	0	27	6	82
Moderate Infections	10	8	80%	2	7	3	70
Severe Infections	19	7	36%	12	6	13	31

Table II contains a second series of 62 cases in which the Schilling count was done in addition to noting the proportion between total count and polymorphonuclears. In column two the uninfected cases, 100 per cent are proportionate, in moderate cases 80 per cent, and in severe infections only 36 per cent. Paralleling these facts, the immature count is within normal limits of 6 in 80 per cent of the clean cases, in 70 per cent of the moderate infections, and 31 per cent of the severe infections. The trend is to increasing disproportion and an increase in immatures as prognosis becomes more serious.

TABLE III

ANALYSIS OF 20 CASES OF APPENDICITIS IN RELATION TO IMMATURE CELLS AND DISPROPORTION

	NO CASES	PRO- PORTION- ATE	DISPRO- PORTION- ATE	IMMATURES LESS THAN 6	IMMATURES MORE THAN 6	PER CENT BELOW 6
Chronic Appendicitis	7	5	0	7	0	100
Acute Catarrhal Appendicitis	4	3	1	2	2	50
Acute Gangrenous Appendicitis	11	3	5	5	3	73

In Table III, twenty cases of appendicitis are grouped. The results show a rather wide variation, and in the individual case, the hemogram would require considerable interpretation and judgment. The marked individual variation emphasizes the point that the whole hemogram must be studied, preferably, in serial counts. In acute gangrenous appendicitis where operation is done immediately and only one hemogram made, all parts of the leucocytic picture must be considered to avoid being led astray.

TABLE IV

CASE A B ACUTE SUPPURATIVE SALPINGITIS

DAY OF COUNT	TEMP	TOTAL COUNT	POLYS	EOS	LYM PHOS	L M	IMMA- TURES	GIB- SON	COURSE
3 12 31	104	22,100	92	0	6	2	11	+5	Very sick
3 13 31	101.2	23,100	90	0	7	3	12	+2	Trifle better
3 14 31	99.8	15,500	79	1	16	4	5	-2	Much improved
3 16 31	100	20,700	84	1	11	4	7	-2	Much improved
3 18 31	99.2	21,200	81	2	11	6	6	-5	Much improved
3 21 31	99	14,300	72	4	17	4	5	-7	Much improved

The use of repeated counts in observing the progress of an infection is shown in Table IV. The high total count with polymorphonuclears increased to plus five units of disproportion indicates a severe infection. Other parts of the hemogram are confirmatory, namely, absence of eosinophiles and increase in immature cells to eleven. Succeeding counts parallel the clinical improvement with a fall in the total count and polymorphonuclears, reappearance and rise of eosinophiles, fall of immatures, rise of monocytes and gradual return of the Gibson index from plus five to minus seven.

COMMENT

The reports of cases in the literature showing high immature counts deal with conditions attended by severe toxemia where there is powerful stimulation of the leucocytic organs. Pneumonia, acute meningitis, acute mastoiditis, and sinus thrombosis are examples. Such toxic states do not occur in average acute abdominal conditions except in neglected cases or cases in extremis. Uninfected cases, of course, have no definite increase of immatures. The value to the general surgeon, therefore, lies in a thorough understanding of the blood picture, to be able to interpret and weave into the clinical picture variations of a few cells.

Our experience in this very small number of cases would lead us to believe that a slight disproportion between total count and polymorphonuclears will often prove of value in turning the shade of opinion in the diagnosis of acute surgical conditions before the condition has advanced to the point where an increase in immature cells commences to show. Menninger and Heim's⁹ study of 1945 cases support this view. The immediate value will depend on the surgeon's detailed understanding of the interplay of the white cells gained from experience in applying the knowledge to individual cases. Routine postoperative counts are valuable in following the course of seriously ill cases and observing complications in clean cases.

CONCLUSIONS

1. In 231 surgical cases studied with the Ehrlich differential count and Gibson index the total count and polymorphonuclears tend to increase with the severity of infection. There is a disproportionate increase of polymorphonuclears to total count in the severe cases.

2. In sixty one cases studied by the Schilling method the immature cells are found to increase with seriousness of the condition.

3. There is a wide individual variation in the Gibson index and Schilling hemogram. All parts of the leucocytic picture should be considered in interpretation, and where time allows, serial hemograms are much more valuable in establishing the trend than a single count.

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THE ACTION OF AVERTIN ON VOLUNTARY AND NONVOLUNTARY MUSCLE*

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AVERTIN was synthesized by Willstätter and Driusburg¹ in 1923 and investigated pharmacologically a few years later by Eichholtz. Shortly thereafter as E 107, it was placed on trial at several German clinics a number of which reported their observations as to its effects on man at the Chirurgen Kongress of 1927.² In the scant five years which have elapsed since, hundreds of papers, clinical and experimental have appeared, dealing with various phases of its action. A review of the existing literature from the anesthetist's standpoint was made by Lundy³ in 1929 and a more complete summary was published by Anschultz, et al.,⁴ in 1930.

The drug, as is well known, is commonly administered rectally as a basic anesthetic. It rapidly penetrates the mucous membranes of the lower bowel, disappearing completely within about fifteen minutes.⁵ Its sojourn in the body, probably in conjugated form, is very lasting, as is evidenced by the prolonged anesthetic effect of one and one half hours,⁶ by its enduring analgesic action of over twenty-four hours,⁸ and the tardy excretion of the products of its decomposition.⁹ With the large doses which are employed (80 to 150 mg per kg), the tension of the drug in the blood stream rises to a high level, the maximum being according to Siebening¹⁰ in from twenty to thirty minutes.

Following the administration of avertin, there have been reported disturbances in the lower bowel and retention of urine rendering catheterization necessary.¹¹ It has been observed in animal experiments that initially, on rectal administration there may be efforts at expulsion, but that these efforts subside even before analgesia is in evidence. The above are suggestive of a depressant effect being produced locally on the rectum and systemically on the bladder and have prompted this investigation of its action on musculature in general.

EXPERIMENTAL PROCEDURES

The avertin (tribrom-ethyl alcohol) used in this report was the pure crystalline substance—not avertin-fluid, which contains amylene hydrate. It was prepared from the latter by evaporation in the dark at 20° C, repeatedly

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washed with distilled water, and dried. The long, glassy crystals thus obtained were preserved in glass stoppered bottles and showed no evidence of deterioration within six months.

The solutions of the drug were always prepared within a few minutes of their intended use. According to the purpose for which they were to be employed, the solvents were sodium chloride, Ringer's, or Tyrode's solutions. The ranges of dilution were from 1:3000 for excised, immersed tissues to 1:40 for direct application to mucous membranes. To facilitate dissolving, the crystals were triturated with portions of the solvent at 40° C until a fine suspension was obtained, this was diluted to the required strength and the preparation was maintained at the above temperature until thoroughly dissolved. Then the material, thoroughly shaken, was brought to the working temperature and retained at that point until used.

Both voluntary and nonvoluntary muscle were investigated. Of the former group, there were examined the erector spinae, gastrocnemius, abdominal oblique and rectus, and sartorius, of the latter, the stomach, small and large intestines, uterus, bladder, ureter, arteries, and heart. All the usual kinds of laboratory animals were employed, i. e., frogs, turtles, rats, dogs, cats, rabbits, and guinea pigs. The effects of the drug were studied on the above tissues in the intact animal as well as in the excised state. The details as to the technique employed were necessarily different for the several tissues and will be presented along with the experimental data.

EXPERIMENTAL DATA

The experimental data can best be presented under captions appropriate to the kind of muscle dealt with or the specific organ from which taken. These will further be subdivided according to the several methods of applying or administering the drug.

1 *Voluntary Muscle*—The effect of avertin on freshly excised voluntary muscle was tried on the gastrocnemius, sartorius, and abdominal rectus and oblique, using frogs, cats, and rats, and on the in situ muscle, using the erector spinae and calf groups of frogs and rats. The drug was dissolved in Ringer's solution for the cold blooded tissue and in 0.9 per cent sodium chloride or, in special cases, sodium chloride of a strength slightly stronger or weaker than 0.9 per cent.

a *Excised Skeletal Muscle* The whole muscle, or strips cut from it, was suspended in a small reservoir of the saline connected for a direct lever tracing, and lightly weighted. Its tone and responsiveness to electrical stimulation (minimal shock, induced current) were recorded. Then avertin dissolved in the same saline was added sufficient to make the desired dilution, or the tissue was transferred to another reservoir containing the desired strength of drug. Particular attention was given to changes in tone, irritability, and contractility.

Effects were obtained with concentrations of avertin ranging from 1:400 to 1:40. In all cases there was a shortening and an increased firmness in proportion to the concentration of the drug. With the higher strengths, this was a definite rigor and resembled closely the phenomenon produced by applying

chloroform directly to a muscle. With concentrations below 1/100 the effects could be abolished and reproduced repeatedly by alternately immersing the tissue in saline and avertin-saline, but with strengths above 1 per cent, it was progressively more difficult to restore the initial condition, while with 1/40 the shortening became maximal within a few seconds and was not removable. The irritability to electrical stimulation was not appreciably altered by 1/100 and lower concentrations but was markedly decreased by higher, no responses being obtainable after the maximal shortening had been reached with a 1/40 solution. The contractility was little affected even by the highest concentrations, i. e., the apex of the muscle curve remained on about the same horizontal line. However, the tracing was otherwise altered in two notable respects: (1) the latent period was lengthened and (2) relaxation was retarded and incomplete, so that the tissue failed to relax to the level at which the contraction started. As a result,

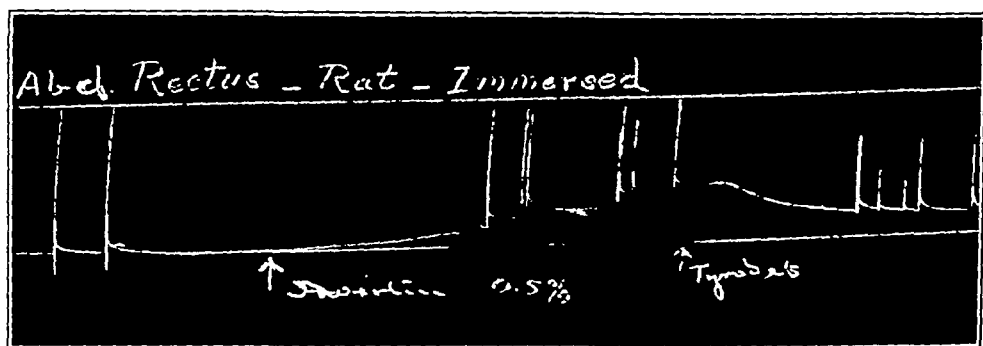


Fig. 1—Shortening of voluntary muscle after avertin. Abdominal rectus (rat, excised, immersed 7 c.) showing shortening produced by 0.5 per cent solution of avertin, the effect of submaximal stimulation, and recovery on removing the drug.

when two stimuli were applied at a short interval apart a stairway-like graph was produced.

While the phenomena were essentially alike after all strengths of avertin, a more detailed study was devoted to those below 1/200 with a view to ruling out possible changes in osmotic tension. Thus baths were used varying not only in the concentration of avertin but also in that of the salts. Various strengths of the drug were tried out in normal saline, in half normal, in twice normal and in three-fourths normal and the results were checked against saline of the same concentration. It may be noted that the osmotic tension of avertin is low in comparison with that of sodium chloride—probably about 1/80 so that 1 per cent avertin in 0.98 per cent sodium chloride is approximately equivalent to 1 per cent sodium chloride. In the strengths employed avertin does not appreciably alter the osmotic pressure of salines. The surface tension of the salines is, however, markedly reduced, and the effects noted above may be in part attributable to this, since the alteration in osmotic tension does not operate. The phenomena resemble water-rigor. In this connection it may be also noted that avertin is an active hemolytic agent. 1 per cent solutions in 1 per cent sodium chloride laking red blood cells almost as rapidly as does distilled water.

b In situ Skeletal Muscle, the drug being applied directly Here the drug was administered by injection into the adjacent lymph sac or into the surrounding subcutaneous tissue. The erector spinae group in frogs and the thigh and calf muscles in frogs and rats were studied. The effective concentrations and the resulting phenomena were almost identical with those observed in the case of the excised tissue. The shortening of the muscles was evidenced by a posture characteristic for the part affected, for instance, opisthotonos after injection into the dorsal lymph sac and toe-drop on injection into the calf of the leg. After concentrations of 1:200, the shortening of the muscle was reducible by the subcutaneous injection of normal saline. One per cent and higher produced a rigor which could not be reduced by the injection of fluids, in fact, in the case of rats following injection of the calf, the leg remained flexed and palpably firmer and the lameness persisted for 24 hours or more. Frogs that

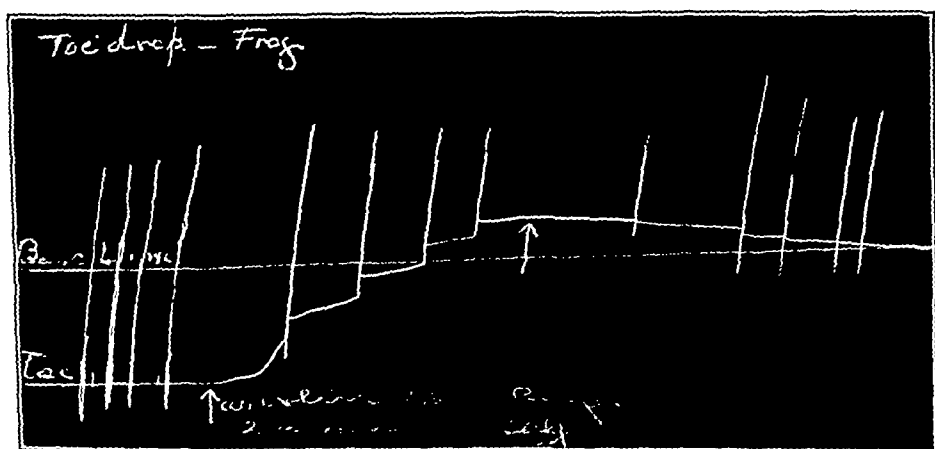


Fig 2—Toe drop after application of avertin to the leg. Calf muscles (frog in situ subcutaneous) showing extension of the ankle produced by 1 per cent solution of avertin and the effect of submaximal stimulation of the nerve.

had received sublethal doses usually recovered their ability to flex ventrally within 24 hours.

c In situ Skeletal Muscle the drug being administered intravenously or rectally The effect of absorbed avertin on voluntary muscle was examined on the leg muscles of the frog and the rat. The former were pithed, the latter, anesthetized with amytal. The arrangement of the experiments was as follows. The knee and ankle were fixed by passing pegs through the respective joints, the foot was flexed and connected with a recording lever weighted just sufficiently to describe a horizontal line. Then the drug was injected into the ventral lymph sac of frogs (30 mg. as a 1 per cent solution in Ringer's fluid per 30 gm. animal) and into the femoral vein of rats (100 mg. as a 1 per cent solution in 0.9 per cent sodium chloride per kg.) or into the rectum (200 mg. as a 2 per cent solution in distilled water per kg.). A moderate reduction in tone was observed in frogs when the spinal cord was intact but with the cord destroyed prior to the administration there was no decrease in tone of the muscles which may be attributed to the tonelessness following spinal pithing. Rats exhibited a decrease

in tone both after rectal and intravenous injection, this was greater after the intravenous which may have been due to the tension of the drug having been raised more rapidly. The relaxation was greater than after the inhalation of ether but much less than after chloroform. In this connection, it may be noted that other observers, using different methods, have stated that avertin systematically effects a relaxation commensurate with that after chloroform.⁵

2 *Alimentary Tract*—The several parts of the alimentary tract examined were the stomach, duodenum, colon and rectum. Both excised segments and in situ organs were studied. The experimental animals employed were cats, rabbits, dogs and rats.

a *Excised Segments*. Freshly excised segments and strips from recently dead animals were suspended according to the Magnus method¹² and immersed in Tyrode's solution. On application of avertin all parts of the digestive tract

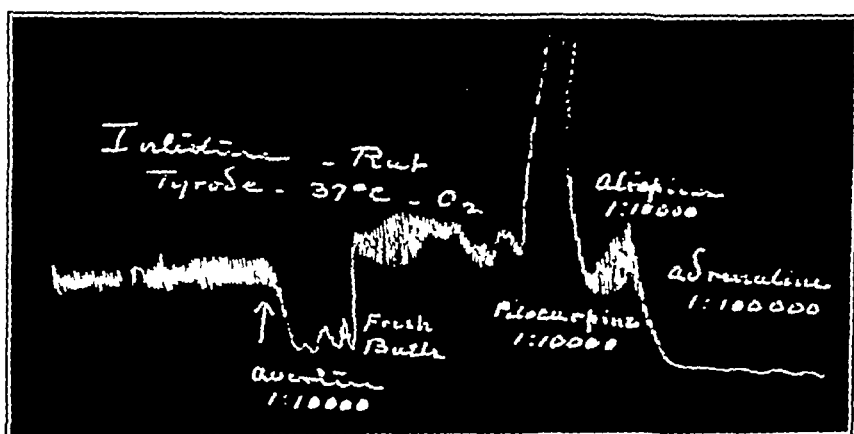


Fig 3—Relaxation of the intestine after avertin. Duodenum (rat excised immersed 37° C) showing loss of tone and decrease in rhythmic activity produced by 1:10,000 solution of avertin and also the actions of pilocarpine, atropine and epinephrine.

exhibited decrease in tone, amplitude of contraction and rhythmicity, in proportion to the concentration of the drug. Effects were observed after 1:3000, these were marked after 1:1500, and all activity was abolished after 1:300. Removal of the drug was followed by prompt recovery of activity. Pilocarpine, atropine, epinephrine, and barium produced their usual effects even in the presence of avertin though these were quantitatively reduced. Pilocarpine and barium were antagonistic to avertin and vice versa. Atropine does not prevent the action of avertin, but, as is also true of epinephrine, it augments the depressive effects on the alimentary tract.

b *In situ Organs, the drug being applied directly*. The parts of the tract examined here were the stomach and rectum, the dog and cat being employed. Balloons connected with recording tambours¹³ were inserted in the organ. These were then inflated with air, so as to moderately distend the viscus. A stomach or rectal tube was introduced along beside the balloon to facilitate the administration of the drug, its inner extremity being placed proximal, distal, or midway, relative to the balloon. The responses to avertin were in every particular like

those reported above for the excised tissue, i. e., the tone, amplitude, and rhythm were decreased by all concentrations. Following the injection of a 2½ per cent solution, the usual concentration employed in man to produce basic anesthesia, there was a total abolition of all activity, the viscus becoming widely dilated with an absence of all contractions. In a few experiments, where hunger contractions were being recorded, it was noted that such were allayed by the introduction of ½ per cent solutions into the stomach. It was further observed that the immediate effect of a rectal injection was an effort at expulsion of the drug—a reflex effect due to the chemical irritation which is operative before the drug has penetrated to the musculature, but after a few seconds these efforts subsided,

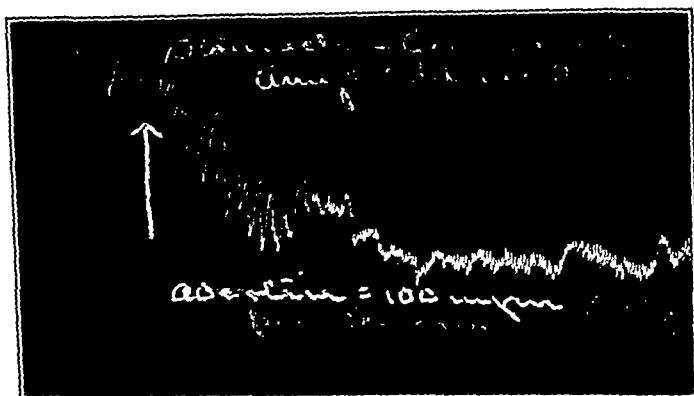


Fig. 4—Depression of the stomach after avertin. Stomach (cat in situ balloon record), showing the effect 100 mg per kg administered through a stomach tube. The small waves which come into evidence as the depression progresses are respiratory.

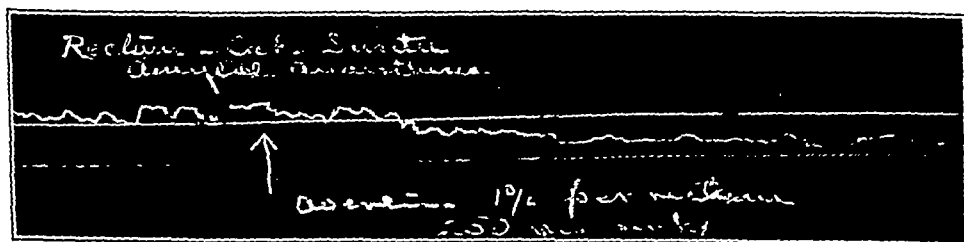


Fig. 5—Depression of the rectum after avertin. Rectum (cat in situ balloon record) showing the effect of a 1:100 solution of avertin administered through a rectal tube.

after which there was occasionally an involuntary voiding due to the relaxed state of the canal.

c. In situ Organs, the drug being administered intravenously or rectally. The stomach and rectum of cats and rabbits were investigated. Balloons were inserted in the organs and the same method of recording was employed as in the above where the drug was introduced directly into the organ. Here, however, the animal was lightly anesthetized with amytal (50 mg per kg, intraperitoneally) and the avertin was administered intravenously for the observation of its effects on the stomach and rectum and rectally for those on the stomach alone. The phenomena produced were so alike those observed on the excised tissue and after direct application to the in situ organ that detailed consideration would be

hardly more than a repetition of what was stated above. In brief, the rectum exhibited decreased activity after 100 mg per kg, intravenously, the stomach, an initial increase in activity, very transient followed by a decrease after 100 mg per kg, intravenously, and after 200 mg per kg rectally. It was noted above that pilocarpine and barium antagonize avertin on the excised muscle of

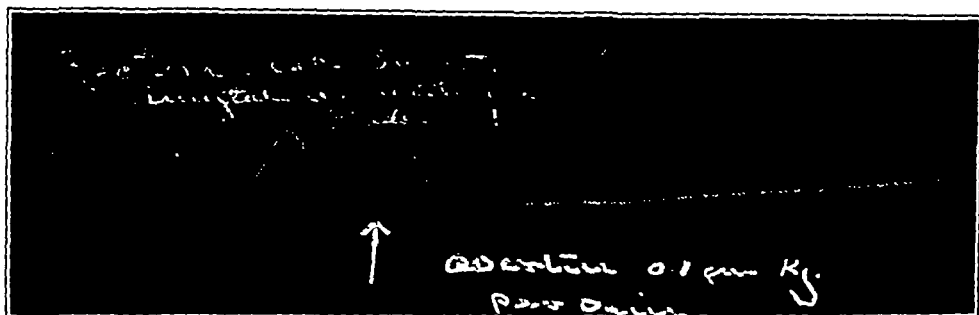


Fig. 6—Depression of the rectum after avertin. Rectum (cat in situ balloon record) showing the effect of 100 mg of avertin per kg, administered intravenously.

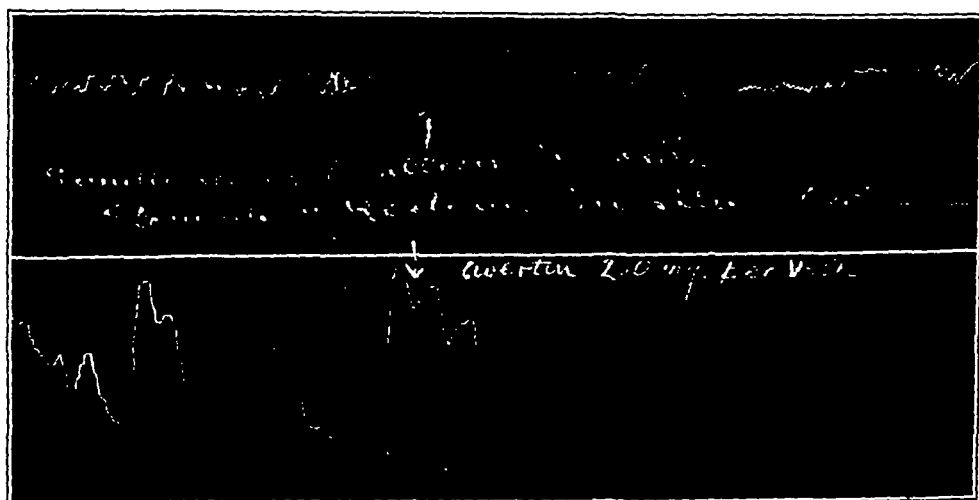


Fig. 7—Initial stimulation of the stomach with subsequent depression after avertin. Stomach and rectum (cat in situ simultaneous balloon records) show a transient initial stimulation of the stomach and depression of the stomach and rectum produced by 200 mg of avertin per kg administered intravenously.

the digestive tract, and it may be observed here that carbon dioxide similarly antagonizes in the intact animal, as was demonstrated by the inhalation of the gas or its accumulation during asphyxia.

3. Ureters. Freshly excised ureters of cats, pigs, and calves were stripped of their connective tissue and examined using suspended sections for observations on changes in rhythm and whole organs for alterations in passage of fluids. The ureter of the pig proved to be the most active.

a. Suspended Preparations. These sections were suspended and arranged for a direct lever tracing, as was done in the case of the alimentary tract. The immersion fluid was Ringer's solution containing 1 gm of sodium bicarbonate

per liter, it having been determined that activity was best exhibited and vitality was longest maintained in this rather than in Tyrode's solution. Only actively contracting preparations of the tissue were employed. On changing the bath to one containing averin dissolved in the same Ringer-soda solution, the tissue almost immediately became quiescent with concentrations as low as 1:2000,

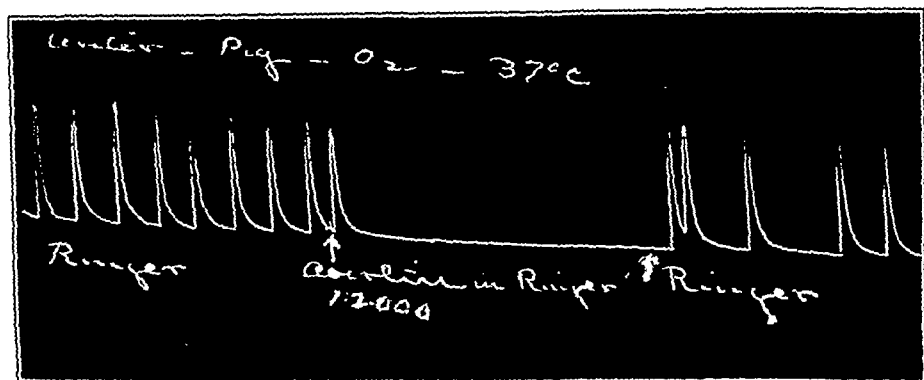


Fig. 8—Relaxation of the ureter after averin. Ureter (pig excised immersed 37°C), showing decreased activity produced by a 1:1000 solution of averin, and recovery on withdrawing the drug.

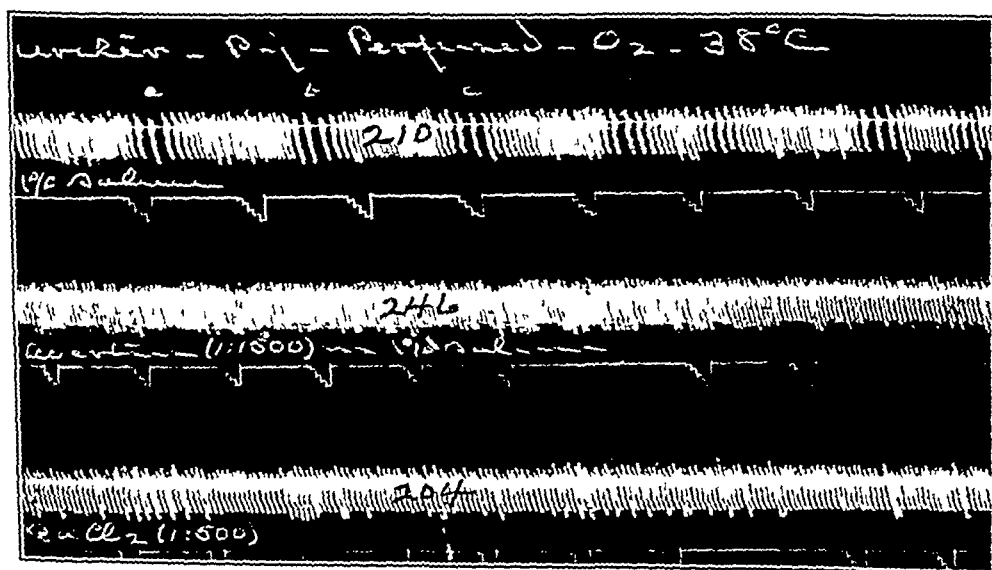


Fig. 9—Dilatation of the ureter after averin. Ureter (pig excised perfused outflow record in drops) showing increase in the flow of the perfusate produced by a 1:1500 solution of averin also antagonism by bitum chloride.

while ones of 1:3000 produced decreased activity. The initial state of the tissue was restored by a change of the bath to the original saline.

b. Perfused Preparations. Cannulas were inserted in the extremities of whole ureters and perfusion was carried on in accordance with the method of Tritton, Wright and Bulow¹¹ the outflow being recorded with a dropcounter of the type described by Briskin and Dan.¹² The perfusate which was admitted

through the kidney extremity of the ureter, was 1 per cent sodium chloride containing 1 gm of sodium bicarbonate per liter. With this solution, the organ exhibited rhythmic contractions and a periodic increase and decrease in outflow. Avertin, 1:1500 and 1:1000 in the above salt-soda solution produced an increase in the outflow, which now became regular. These changes were presumably due to the inert, tubelike state effected in the passageway, as may be deduced from the action of avertin on the suspended ureter. Return to the initial peristalsis was followed by a decrease in the flow, but the rhythmic activity was not restored. Pilocarpine and barium chloride antagonized the effects of avertin, both on the perfused and immersed ureters.

1. Urinary Bladder. As in the case of other organs experiments were performed on the excised and intact urinary bladder. The drug was applied to the excised organ directly and through the blood stream.

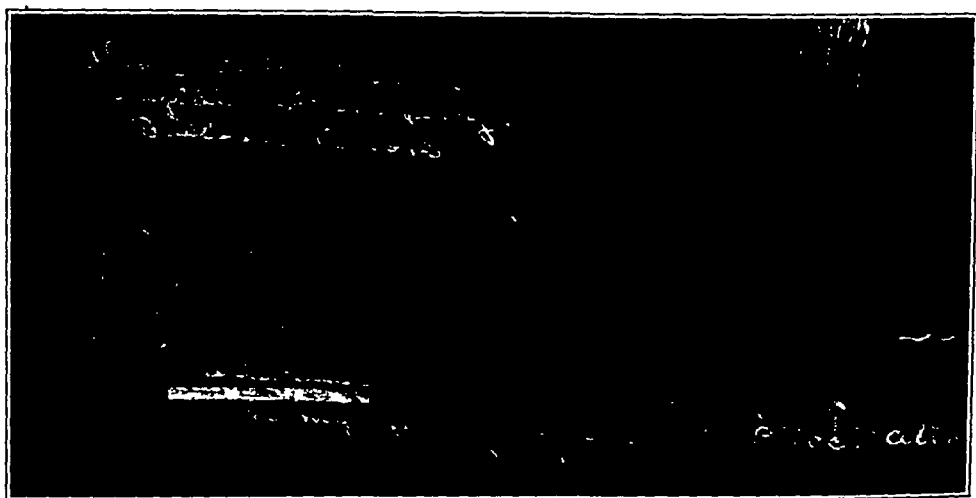


Fig. 10.—Relaxation of the bladder after avertin. Bladder (cat in situ balloon record) showing the suppression of activity produced by 100 mg of avertin administered intravenously also antagonized by pilocarpine.

a. Excised Sections. Strips from the bladder were suspended and immersed as was done in the case of the parts of the digestive tract. Tyrode's solution was used in the bath, only active preparations of the tissue were employed. On application of avertin, depressive effects were exhibited similar to those observed on the intestine, i. e., tone, amplitude and rhythmicity were decreased with weak strengths (1:2000) and all activity was abolished after strong (1:800).

b. In situ Organ, the drug being applied directly to the mucous membrane. The results here corresponded with those reported for the immersed sections.

c. In situ Organ the drug being administered intravenously. Cats were given amytal sufficient to produce the degree of basic anesthesia and whiffs of ether were supplemented as necessary during the operative procedures. An incision was made just above the symphysis pubis, a balloon connected with a tambour was inserted in the bladder, and the wounds in the viscus and body wall were sutured. The balloon was then moderately distended and the tracing be-

gun. Within a few minutes, rhythmic contractions and tone changes were in evidence, on which were superimposed respiratory and pulse waves. Avertin (50 mg per kg, intravenously) diminished the tone and tonus waves without appreciably affecting the rhythm. The respiratory waves became more marked, probably due to the lowering of the tension in the balloon following the decrease in tone of the viscus. After 100 mg per kg, there was a cessation of all activity. The organ was completely relaxed, but could still be excited by parasympathetic stimulants (pilocarpine) and muscle stimulants (barium), though doses larger than usual were required.

5. *Uterus*. The same general lines of experimentation were carried out in the case of the uterus as in that of the preceding tissues and organs. Cats, dogs, rats, and guinea pigs were employed.

a. *Excised Segments*. Sections from the various uteri were suspended and

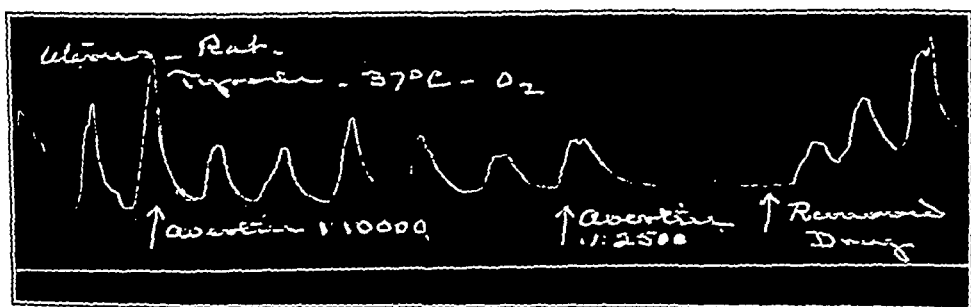


Fig. 11.—Depression of the uterus after avertin. Uterus (rat excised immersed 37° C) showing decrease in tone produced by 1:10,000 solution of avertin and quiescence by 1:2500, also recovery on removing the drug.

immersed in Tyrode's solution and the same procedures were instituted as in the case of the excised intestine. The organ proved to be very reactive to avertin, effects being in evidence after dilutions as low as 1:3000. These consisted in a reduction in tone, height of contraction, and rhythmicity. Strengths of 1:800 produced complete quiescence. Washing the drug out, after a few minutes contact, restored the tissue to its initial activity, rather tardily however following the high concentrations.

b. *In situ Organ, the drug being administered intravenously*. Rats were employed here. The animals were lightly anesthetized with amytal and given whiffs of ether during the operative procedures. The abdomen was opened with scissors and its edges were raised so as to form a cuplike receptacle, which was filled with physiologic saline at body temperature. The vaginal extremity of the organ was immobilized by means of a pointed rod fixed to a stand and the ovarian end was attached to a recording lever by means of a hook and thread. The latter was passed around a pulley-wheel so that the organ could be maintained approximately in a horizontal position. Care was exercised throughout not to disturb the position or circulation of the contents of the abdominal cavity. With the above arrangement the rat's uterus usually exhibits active movements, consisting in rhythmic contractions and moderate but irregular changes in tone. Avertin, administered intravenously, produced a decrease in activity, first of

the tone, then of the amplitude and rate of contraction. Doses of 150 to 200 mg were followed by quiescence, while those of 50 to 75 mg per kg were usually only mildly depressant.

6 *Heart* Three kinds of experiments were performed with the heart muscle, (a) strips from the ventricle were immersed (b) excised hearts were

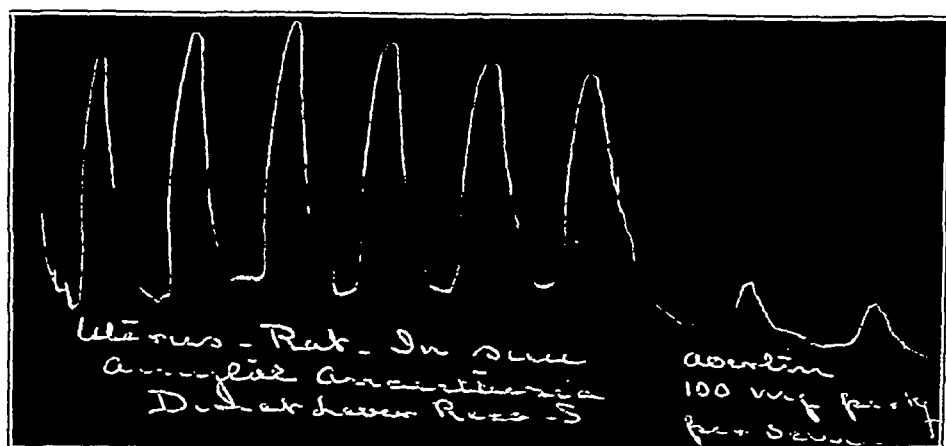


Fig 12—Decrease in uterine activity after avertin. Uterus (rat in situ lever record), showing decrease in tone and contractions produced by 100 mg of avertin administered intravenously. The small waves are respiratory.

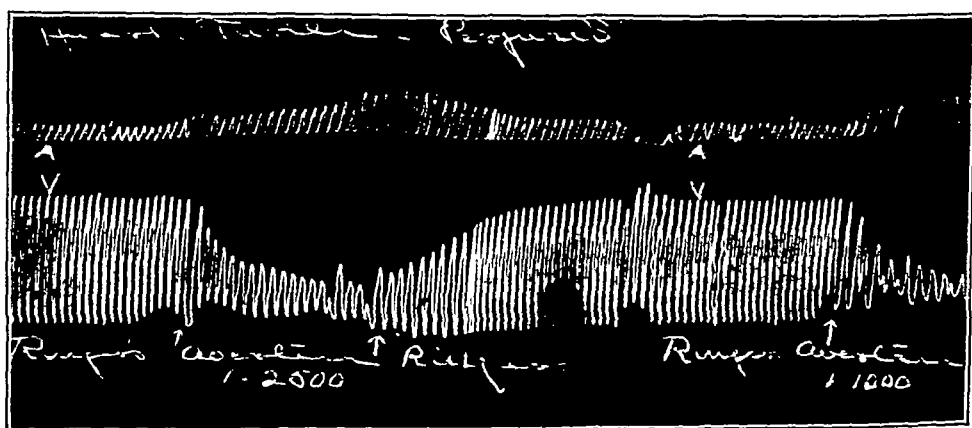


Fig 13—Depression of the heart after avertin. Heart (turtle excised perfused apical record) showing decrease in systole and diastole and slower rhythm produced by avertin.

perfused and (c) in situ hearts were treated through the normal blood stream. Turtles, dogs, and rats were used.

a *Immersed Strips of the Ventricle* These were cut from the heart of the turtle and immersed in Ringer's solution containing 1 gm of sodium bicarbonate per liter. After regularity in contractions had been recorded avertin dissolved in the same saline was applied. This produced the same picture of depression noted for other tissues. Solutions of 1:2500 decreased the activity, those of 1:1500 produced quiescence. In both cases it was possible to restore the tissue

in tone, amplitude, and rate by washing out the drug. The same results were obtained at 30° C. as at room temperature.

b Perfused Hearts. The hearts of turtles were perfused with Ringer's solution, employing a method previously described.¹⁶ The perfusate was admitted through the vena cava, the perfusion pressure was maintained constant, the record was taken from the apex of the ventricle. After the cardiac activity had become uniform, avertin dissolved in Ringer's solution was admitted. The effects observed were the same as in the case of the excised strips. The organ in the perfusion experiments was, however, a little more reactive, in that now 1:3000 depressed and 1:2000 produced quiescence. Both auricle and ventricle were affected, block was never in evidence. Resuscitation was readily effected by perfusion with Ringer's solution and by administering epinephrine. There was not shown the increase in force, preceding the depression, which has been described for the rabbit's heart by Parsons.⁸

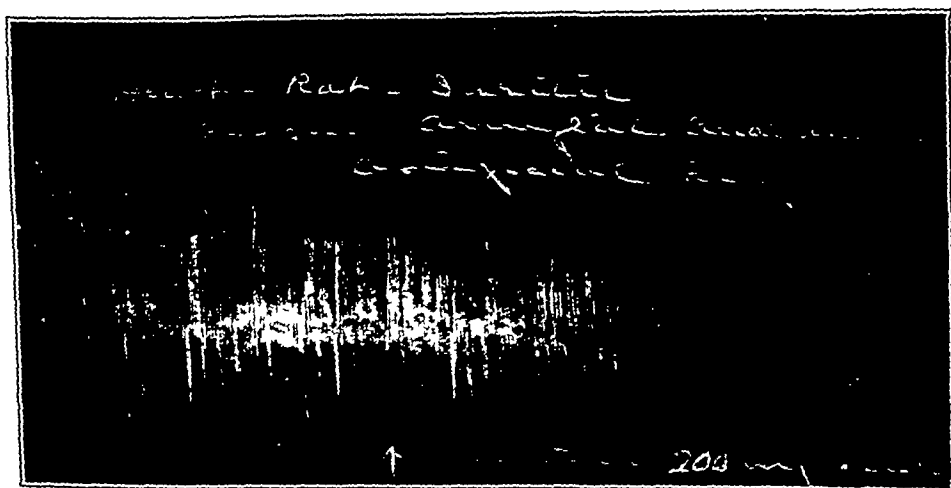


Fig. 14.—Cardiac depression after avertin. Heart (rat in situ, apical record) showing decrease in pulse volume produced by avertin 200 mg per kg administered intravenously.

c In situ Hearts, the drug being administered intravenously. These experiments were of two kinds: (I) ones performed on rats specifically for the cardiac effects, and (II) ones on dogs done in the course of a study of the changes in blood pressure.

I Rat's Hearts. Amytal was administered to rats in quantity sufficient for basic anesthesia, whiffs of ether were supplemented during the operative procedures. A cannula was inserted in the femoral vein, artificial respiration was instituted, and the apex of the heart was exposed through a small window and connected with a recording lever. After hemorrhage had been controlled, a few cc. of normal saline were injected into the vein to make good the loss of blood during the preparation of the animal. Care was observed to maintain the body temperature. With the above technique the rat's heart is an excellent object for study. The intravenous administration of less than 100 mg per kg produced no appreciable effect, but those of 200 mg per kg, which is less than

the anesthetic dose for rats, markedly reduced the pulse volume by decreasing both systole and diastole. There were not exhibited the preliminary quickening described by Parsons³ nor the initial diastolic depression of Anshultz and Specht.⁴

II Dog's Hearts Dogs were anesthetized with amytal and ether or with morphine and ether. A cannula was inserted in the femoral vein and a rectal tube was secured in position so that either the venous or rectal route of administration could be employed without disturbing the animal. The pulse and blood pressure tracings were taken from the carotid, all observations being made under uniform depths of anesthesia. No cardiac changes were observed after rectal doses of as much as 300 mg per kg, nor after 150 mg per kg by vein, if administered very slowly. It may be noted that dogs are highly resistant to

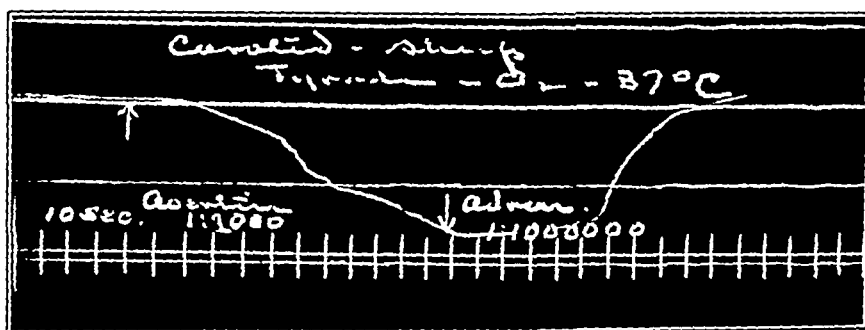


Fig 15—Depression of arterial muscle after avertin. Carotid (sheep excised spirals immersed 37° C) showing relaxation produced by a 1:2000 solution of avertin and the antagonistic effect of 1:1,000,000 solution of epinephrine.

avertin, the rectal dose for basic anesthesia being about 500 mg per kg and the intravenous about 150 mg per kg. However a 100 mg dose per vein given rapidly or two 100 mg doses at 15 minutes apart produced a slight decrease in the pulse volume, followed by a corresponding fall in the blood pressure. The heart recovered within from fifteen to twenty minutes. Further administration of the drug introduced complicating blood pressure changes, rendering interpretation difficult from the standpoint of the heart alone, hence, the effects of increasing the dosage will be dealt with in connection with the data on the vascular system. It may be noted here however that no dosage of avertin employed altered the cardiac effect of electrical stimulation of the vagus nerve and that pilocarpine, atropine, and epinephrine still produced their usual actions on the heart.

7 Arteries The effects of avertin on the arterial system were investigated by three methods, (a) on excised vessels, (b) on perfused tissues, and (c) on the intact animal.

a Excised Arterial Strips Spirals of several circumferences in length were cut from the carotids of sheep. Prepared in this manner, the tissue consists essentially of circular fibers.¹⁹ These strips were then immersed in oxygenated Tyrode's solution at body temperature and connected with a recording lever as in the case of the intestine. The lever was weighted for ten minutes to effect

decrease in tone, after which the weighting was so adjusted that a horizontal line was recorded. On introducing avertin dissolved in the same saline, the artery exhibited a relaxation, which was moderate and gradual with 1:2000, but profound and sudden after 1:1000. The former could be counteracted by epinephrine 1:1,000,000, the latter by 1:500,000. Even washing alone restored the tissue after the lower concentrations of avertin, so that the effect could be produced and removed several times in succession. Accordingly, it does not appear that the drug inflicted any damage outlasting its sojourn in the arterial wall.

b Perfused Vessels. Frog's vessels were perfused with Ringer's solution by the Fuehner method,¹⁰ the outflow being recorded with a drop counter of the kind employed with the meter.¹¹ After adjustments had been made so as to secure a uniform rate of perfusion, avertin 1:2000 in Ringer's solution was intro-

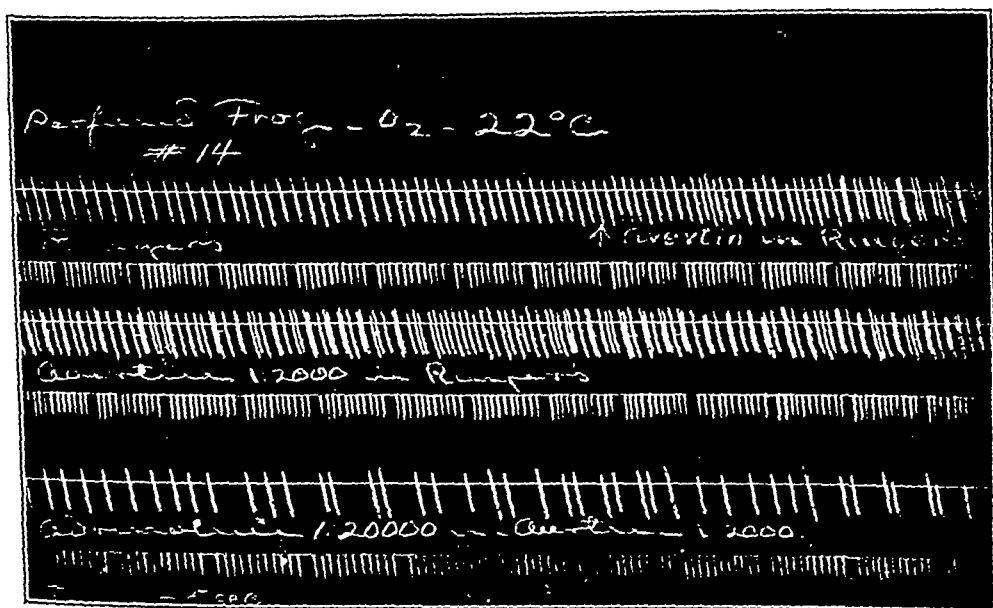


Fig. 16—Vasodilatation after avertin. Vessels (frog's body perfused 20°C) showing increased rate of perfusion produced by 1:1,2000 solution of avertin and antagonism by epinephrine.

duced. The outflow gradually increased, in some cases by as much as 40 per cent, demonstrating a marked vasodilatation. This could be antagonized by epinephrine but it was necessary to employ massive doses. In connection with these experiments it is interesting to note that Parsons¹² perfusing the rabbit's leg noted a decreased outflow while Raginsky, Bourne and Bruger¹³ reported an increase in the coronary flow in their work with heart-lung preparations.

c Intact Animals. The blood pressure tracings were made in connection with the study of the effect of avertin on the heart and the experimental procedures were outlined in the paragraph dealing with that topic. The rectal administration of 300 mg per kg of avertin produced no alteration in the blood pressure; the same was true following 100 mg per kg by vein if admitted very slowly and is a 1 per cent solution in normal saline. But when injected rapidly,

there was a "shock" effect in which a decrease in pulse volume preceded the fall in arterial pressure, recovery ensued within a few minutes. Two slow injections of 100 mg per kg at fifteen-minute intervals, produced a gradual drop in pressure of 10 mm from which there was recovery in twenty minutes. The heart was seen to contribute, in that while the rate was not changed the amplitude of the pulse was decreased (the opposite to what would be the case when the vessels are dilated). A third dose now of 100 mg per kg was followed by a gradual decline in pressure, occasionally, even to zero. Death resulted in some cases from respiratory failure, in others, from circulatory collapse, which was vascular, in that the heart continued to pulsate after the arterial pressure had become zero.

The question of the blood pressure changes has received a great deal of attention in literature. Bender¹⁷ concluded from his experiments that they were due to decrease in vasomotor tone, Parsons,⁸ that they were cardiac, and Ragsinsky, et al¹⁸ that they were both cardiac and vascular. The conclusions reached in my investigation are that they may be cardiac, vascular, or cardiovascular. Different species differ as to the susceptibility of the several parts of the circulatory apparatus to avertin, and different individuals of the same species, probably due to their health status, likewise vary in their responsiveness, for instance, well-nourished animals and gorged patients tolerate larger doses as a result of their increased powers for detoxicating the drug.

Other observers^{5, 18} have reported that epinephrine can restore the blood pressure in an avertin depressed animal, this has been confirmed in my work—but large doses are necessary in the intact animal as was also shown on the excised arterial strips and in the perfusion experiments. Hence, the antagonism of epinephrine does not exclude the presence of a direct depression of the vessel wall. The fact that there is depression of the respiratory center, which is the most usual cause of death, suggests a depression also of the contiguous vasomotor center. This is no doubt the primary factor in some cases and only a contributory one in others. In treating the convulsions produced by strychnine it was noted that avertin produced a decrease in blood pressure even before the tetanic spasm was abolished and that it diminished the reflex effect on blood pressure following peripheral stimulation in animals which have received a dose of strychnine just under the convulsant one. These would seem to be instances of a depression of the reflexes which are effective through the vasomotor center. It is tenable, moreover, in view of the effect of avertin on the reflexes in general.

SUMMARY

1 Experiments have been presented showing that avertin manifests a definite action on both voluntary and nonvoluntary muscle.

2 Applied directly to voluntary muscle it decreases the irritability and produces a rigor-like phenomenon.

3 After absorption into the blood stream it does not affect skeletal muscle substance directly, but effects relaxation through nervous depression.

4 Avertin depresses all kinds of nonvoluntary muscle both on direct application and after absorption into the blood stream.

5 The depressant effects in the intact animal are exhibited more uniformly and more acutely on the bladder, rectum, and uterus

6 Avertin does not qualitatively alter the reactions of tissue to nerve and muscle stimulants and depressants

CONCLUSIONS

1 The rigor-like effect of avertin on voluntary muscle is a direct action and resembles water rigor

2 The depressant effects of avertin on nonvoluntary muscle are direct actions, affecting the muscle substance

3 The changes in blood pressure may be due to depression of the arterial muscle, the vasomotor center, or the heart, or to a simultaneous depression of two or more of these

4 No permanent damage is produced in tissue even by high concentrations of avertin, resuscitation being prompt on withdrawing the drug

I wish to express my appreciation for the assistance rendered by Dr D D Brime in the experiments on the stomach and the cardiovascular systems

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THE COMPETENCY OF THE REHTUSS TUBE AS A COMPLETE EVACUATOR*

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THE degree of efficiency of the Rehtuss tube as to total gastric aspiration is still unsettled. Usually a small residuum is of no importance, but since the unaspirated quantity is not known and is variable, erroneous deductions could easily be obtained in investigative procedures. We have endeavored by means of a sodium bromide solution and the X-ray to demonstrate the extent of failure of withdrawal of the total gastric content by the tube, and incidentally to determine if the use of the lateral posture is more efficacious than the sitting one in gastric evacuation.

The literature is very meager as to investigative efforts in regard to the residuum after aspiration with the Rehtuss type of tube; however, most writers apparently believe this procedure adequate. Sahli (Potter)¹ referring to the old style tube with several openings at the lower end, states, "By use of the special perforated stomach-tube we are now able to express the contents of the stomach completely." Rehtuss, Beigelm and Hawk² in considering the residuum after fasting, using the Rehtuss tube make this statement, "The method of examination by means of the new modified stomach tube is the only satisfactory method of determining the complete residuum." Reginald Fitz³ also expresses confidence in the sufficiency of the tube. There is likewise little in the literature in regard to the most favorable position of the subject for obtaining the best results in gastric evacuation. Sahli (Potter)¹ states, "The food is usually expelled better if the patient be in the right or left lateral, rather than in the sitting, posture," and further suggests the following maneuvers, "The contents of the stomach are now expressed in the usual way, with the patient lying on the left side. As soon as the flow has ceased the examiner grasps the patient by the shoulders, shakes him well, and holds him over the left side of the bed or table, still retaining the left lateral posture, and depresses the head and trunk until the external end of the tube is lower than the cardia or the epigastrium."

Rehtuss, Beigelm and Hawk² write, "Suffice it to say that we aspirate while the subject is on his back, on his stomach and on each side and is breathing deeply." Bloomfield and Keeter⁴ express the following opinion, "Our impression is that by thorough suction applied with subject on right and left sides and in the dorsal position, all free fluid in the stomach can be withdrawn."

Before the use of the bromide solution a bread baked with one third barium was tried as a test meal, but the barium separated from the bread during digestion and clung to the gastric mucosa, giving a shadow out of proportion to the actual quantity present. However, 90 c.c. of a 7½ per cent sodium bromide solu-

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tion, injected into several emptied fasting stomachs, gave satisfactory x-ray plates and was rather easily removed on aspiration.

Twenty apparently normal patients, who were not obese, were subjected to the following procedure: aspiration of the fasting stomach, withdrawal of the tube, mastication of ordinary bread, reinsertion of the tube, injection of 90 cc of a 7½ per cent sodium bromide solution, the taking of an x-ray film, second aspiration after about eight minutes, and taking of a second film. Eleven of these patients were aspirated in the sitting posture and the other nine in the two lateral positions as well. In the latter series the tube was inserted up to the 75 cm mark and the patient was turned on the right side, and it was gradually withdrawn to the 50 cm mark, with suction applied constantly, as the subject



FIG. 1

was turned from side to side. This procedure was repeated until no further gas or fluid content was obtained.

Of the twenty patients aspirated after a meal of bread and bromide solution as described, only the stomachs of two patients were completely empty, but in none did we find over about 10 cc. In sixteen of them only about 5 cc of residue remained and eleven of these sixteen showed even less than 5 cc. The use of the lateral position does not offer any advantage over the sitting posture, as the two groups give practically the same findings, in fact very slightly better results were obtained when only the sitting posture was used.

For the purpose of estimating the amount of sodium bromide solution in a stomach by means of the x-ray, patients were given varying amounts of the bromide solution after aspiration of the fasting stomach. In these specially selected patients we believed that any quantity in excess of 6 cc gave a definite



Fig 2



Fig 3

x-ray shadow Fig 1 shows a stomach after the injection of 5 c c of a 7½ per cent sodium bromide solution The large air bubble in the cardia of the stomach resulted from the injection of an necessary to push the small quantity of fluid into the stomach Fig 2 illustrates a stomach after bread was eaten and 90 c c of the bromide solution was injected Fig 3 shows the same stomach after emptying

SUMMARY

A residuum of only about 10 c c , the maximum amount found in any case, is ordinarily a negligible quantity, and when we consider that sixteen out of twenty had only about 5 c c and eleven of these even less than 5 c c , the efficiency of this procedure for clinical purposes and for practically all investigative efforts is quite evident Unfortunately, however, at the present time there is no way of determining the degree of completeness of aspiration after any of the food test meals The seemingly strange result of being slightly more successful when only the sitting attitude was employed, can be explained when we consider the fact that the stomach of one patient in the three positions was of the vertical type and very low Obviously in such a case the previous knowledge that the stomach was unusually low would have aided the chances of complete evacuation, as it would have been necessary to insert much more than the usual length of tube This patient had a residuum of about 10 c c Granting that it contained less than 5 c c , the most frequent finding, then the slight advantage would be with those in the lateral positions Since the results in the two groups are so close, it seems that the success of withdrawal of gastric content depends on such factors as the anatomic position of the stomach, spasm of the stomach (as mentioned by Bloomfield and Keefer⁴), appropriate meal and effective suction apparatus, etc , rather than on the posture of the subject

CONCLUSIONS

- 1 With careful aspiration of a bread and water meal the residuum in most cases will be less than 5 c c and not over about 10 c c in any case
- 2 There is no advantage in the use of the lateral posture in the removal of gastric content

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LABORATORY METHODS

ENUMERATION OF PARASITES IN THE BLOOD OF MALARIAL PATIENTS*

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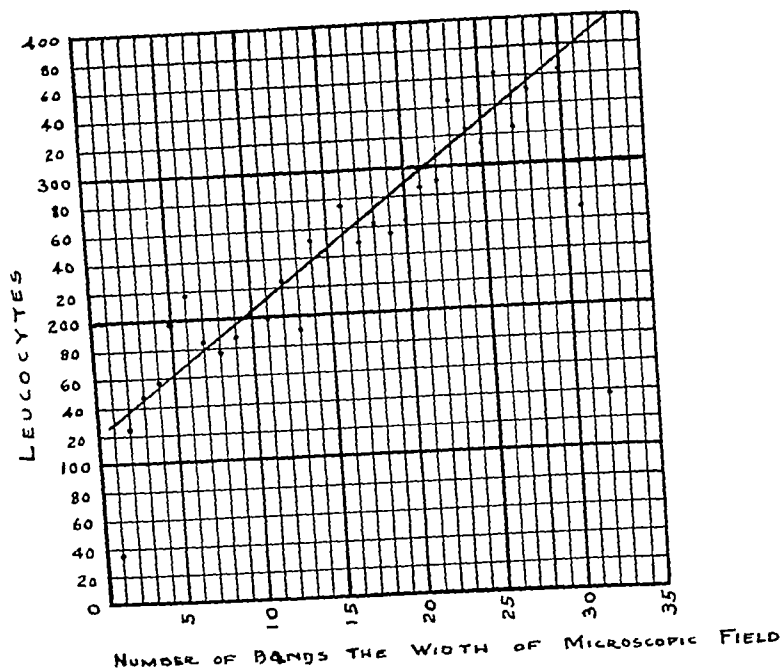
SINTON¹ reviews the methods which have been used for counting malaria parasites in the blood and describes a method of his own which appears to have been adopted by most investigators making any attempt at enumeration. Although his method is practicable and is adapted to field work, the principle of the Thomson method² appeals to us more. This method requires only two or three accurately graduated pipettes, which can be used over and over, no standard suspension of fowl cells is needed, a larger amount of blood can be conveniently examined in a shorter time than by other methods, and in making the counts one is enumerating only parasites and not corpuscles in addition, as is the case when Sinton's methods are used.

The objections which Major Sinton raises to Thomson's method are logical but not difficult to overcome. The first objection, (a) "that a considerable amount of skill is necessary in its use, and the accuracy of the technique may be interfered with by the difficulties which attend the taking of blood from persons under tropical conditions, especially in the case of children" applies to all methods, and it is no more difficult to draw blood to a definite mark in one method than in another. Objections "b" and "c," referring to the small caliber of the pipette, can also be overcome. Our experience has indicated that it is possible to examine approximately $\frac{1}{2}$ cu mm of blood in a thick film in about ten minutes the time we had been accustomed to have technicians spend in survey work. More recently only five minutes has been spent the time necessary to examine $\frac{1}{4}$ cu mm of blood. A pipette for handling that amount of blood must have a small bore, but it was found that 5 cu mm could be handled very well in pipettes on the market which were graduated in cubic millimeters. In these the column length of 5 cu mm of blood is about 10 mm, or approximately the column length of $\frac{1}{8}$ cu mm of blood in the Thomson pipette. The bore is large enough to permit easy entrance of the blood and rapid cleaning. The pipettes as found had too blunt a point to handle such a small amount of blood. They were cut into small sections just long enough to handle 10 cu mm of blood, and one end was drawn out to a fine point the opening of which would admit the finest wire we had for cleaning and still was large enough to permit easy and rapid handling of the blood and satisfactory cleaning. In

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case the glass was too thick at the point it was easily ground down to a slender point on fine sand or emery-paper. The pipette is not graduated to deliver 5 cu mm at the tip, but usually the last mark not affected in drawing out the point was used as zero, and 5 and 10 cu mm marks were made with this as a base.

The method of using the pipette is merely a modification of Thomson's technic to accommodate the larger amount of blood used. The blood is drawn up to the 5 cu mm mark (or to the 10 cu mm mark if duplicate smears are to be made) and without wiping the point unless an excessive amount of blood



MEAN = 243

Sigma (of the distribution) = ± 79 with trend

Sigma (of the distribution) = ± 32 without trend

Fig. 1.—Diagram of duplicate leucocytic counts on two blood smears

has stuck to it one spreads the blood over an area 3 by 15 mm square which in our experience has given a smear which stains uniformly well with Giemsa. The area can be varied however to suit one's convenience. To facilitate as uniform spreading as possible an area of this size is drawn on paper and the slide placed over the drawing. One can easily cover this area exactly by following the diagram underneath the glass slide. The amount of blood is large enough to be spread with the point of the pipette and when the area has been covered the blood is blown out exactly to the zero mark. It is felt that the same amount of blood will stick to the point of the pipette upon finishing as was present when beginning. The rest of the blood can be used for a thin film. The pipette can be cleaned by repeatedly drawing in and blowing out clean

water from a large container. The water in this container can be changed as often as necessary. The time required for the smear to dry depends upon the weather. The slide should be kept as level as possible until at least coagulation takes place. It was felt at first that the pipette should be dried with alcohol and ether, but in practice it has been found sufficient to wash it thoroughly with water and to draw the excess water out with the breath. In drawing up the next sample the pipette is washed by the first portion of the blood column so that the last portion of blood drawn in is quite undiluted by any water remaining on the side of the pipette. It is this last portion that is delivered to the slide for the counting, and the first portion remains between the zero mark and the point of the pipette. If a pipette becomes partially or completely clogged it is placed in water for a time and can be easily cleaned out with a wire. It has never been necessary to use more than two or three pipettes in completing an examination of 400 to 500 blood smears. The pipette can be cleaned before the next survey is started. With a little practice an

TABLE I

DIFFERENT LEUCOCYTE COUNTS ON TWO BLOOD SURVEYS

Number of leucocytes in each band the width of the microscopic field. Every fifth band was counted

	1		2	
	A	B	A	B
1	34	27	45	59
2	124	84	126	138
3	147	165	180	178
4	159	160	199	212
5	196	211	196	182
6	216	199	184	224
7	151	188	199	184
8	175	174	233	223
9	193	201	205	203
10	203	197	212	211
11	197	192	227	197
12	222	221	209	237
13	188	184	214	261
14	252	247	194	206
15	245	259	216	250
16	277	294	254	260
17	248	250	245	280
18	260	260	265	274
19	256	276	293	323
20	261	243	302	259
21	286	270	304	274
22	288	321	294	306
23	346	317	313	296
24	325	345	332	323
25	316	338	334	314
26	361	306	326	350
27	322	296	335	337
28	352	354	339	341
29	381	348	379	294
30	363	345	277	275
31	267	242	190	161
32	138	109	21	
TOTAL	7779	7623	7622	7632

assistant can take the smears as rapidly as the physician can examine individuals for enlarged spleen, which is satisfactory time for most survey work.

There is the possibility that a few parasites may be carried over from a heavily infected blood sample to the next slide when the same pipette is used over and over again. However, series of two or more consecutive positives have not occurred more often with this method than with those previously used, and there have been numerous instances where the slide following one with high counts has been negative.

The method of enumeration employed in our survey is that advocated by Thomson. A square field is necessary for the counting. A special eyepiece can be obtained in which the diaphragm has a square opening instead of the usual round one, or an adequate substitute may be made by cutting a square hole in a circular piece of paper which will fit into the ordinary eyepiece. Too large a field makes counting of heavy infections difficult while if the hole is too small much time is consumed in the counting of light infections. We usually have, therefore, two sets of paper diaphragms, one with a large hole for use in counting light infections and one with a small hole to be used when counting heavy infections. Beginning at one end of the smear the observer counts all the parasites found in contiguous fields as he moves from one side of the smear to the other. This gives the parasites in a band of the smear the width of the microscopic field. For routine work every fifteenth such band across the smear is counted, and as 5 cu. mm. of blood is taken, the number of parasites counted will be that in $1/15$ of 5 cu. mm., or in $1/3$ cu. mm. of blood. If counts begin to run very high, fewer bands are counted, but if a thorough search is to be made, as for diagnosis, more bands can be examined.

TABLE II
DUPLICATE LEUCOCYTE COUNTS ON THE SAME SMEAR

	FIRST COUNT	SECOND COUNT
1	7,000	6,400
2	7,700	7,500
3	6,800	6,400
4	6,100	6,200
5	6,400	5,500
6	9,200	9,000
7	8,500	8,800
8	9,600	9,600
9	9,100	9,900
10	10,000	10,100
11	10,900	11,500
12	10,000	10,700
13	8,300	9,000
14	9,100	9,200
15	7,600	8,100
16	19,200	19,700
17	17,700	18,900
18	18,300	18,300
19	18,400	17,300
20	3,200	2,900
21	2,600	2,500
22	2,600	2,900
23	2,600	2,600
24	2,900	3,000

TABLE III
LEUCOCYTE COUNTS ON DUPLICATE SMEARS

	FIRST SMEAR	SECOND SMEAR
1	7,700	7,900
2	6,500	5,600
3	5,500	4,800
4	6,100	5,700
5	5,900	5,300
6	11,200	11,500
7	4,000	4,200
8	10,400	11,000
9	9,100	8,500
10	7,600	5,400
11	5,200	4,500
12	7,600	5,700
13	8,100	7,000
14	2,800	2,500
15	5,000	4,800
16	2,700	2,600
17	6,500	6,000
18	5,400	6,300
19	3,900	3,400
20	5,900	6,800
21	6,800	6,700
22	2,500	1,300

TABLE IV
LEUCOCYTE COUNTS COMPARED WITH THOSE OF HEMOCYTOMETER

	HEMOCYTOMETER	DIRECT COUNT OF 15 CU MM
1	9,300	7,000
2	8,700	9,000
3	10,300	10,900
4	21,900	19,200
5	3,800	3,200
6	4,200	6,700
7	8,300	7,600
8	3,400	2,600
9	4,300	4,900
10	7,600	6,200
11	4,900	5,700
12	3,200	3,300
13	12,400	12,300
14	2,800	3,700
15	5,900	6,200
16	7,800	5,900
17	7,200	3,600
18	5,400	6,500
19	7,200	6,700
20	2,900	1,900
21	9,800	10,700
22	4,200	6,500
23	7,200	6,700
24	4,500	5,800
25	12,100	10,200
26	7,600	8,500
27	9,500	10,200
28	1,900	1,400
29	4,400	4,800
30	6,700	6,300
31	7,900	9,400
32	6,200	4,600
33	6,100	5,500
34	7,200	7,700

With the use of the oblong smear more bands can be conveniently examined and thus any unevenness in the outline of the smear will be compensated for, though as a rule such a small amount of blood is found in the actual margin that there is very little error.

It may happen at times that the slide is tilted slightly before it is dried, so that the blood runs a little to one end. This is not a serious matter, for the gradation in thickness from one end to the other is very uniform, as is

TABLE V
COUNTS OF MALARIA PARASITES MADE ON THE SAME SMEARS BY TWO MICROSCOPISTS

NO	TOTAL PARASITES PER (1 MM	
	MICROSCOPIST NO 1	MICROSCOPIST NO 2
1	28,188	48,048
2	56	64
3	104	56
4	6,298	7,227
5	4,972	3,962
6	3,360	3,640
7	196	182
8	316	324
9	162	162
10	8,124	8,916
11	264	298
12	2	2
13	4	6
14	0	2
15	0	2
16	10	2
17	28	24
18	16	0
19	88	90
20	14	38
21	7,976	9,504
22	1,110	1,628
23	154	440
24	22	4
25	200	132
26	602	360
27	6	32
28	110	154
29	12	4
30	86	96
31	8	12
32	840	948
33	28	2
34	16	2
35	16	16
36	8,004	7,168
37	1,503	1,704
38	74	92
39	36	38
40	98	156
41	42	76
42	128	108
43	234	264
44	316	460
45	532	348
46	64	34
47	20	10
48	80	50
49	1,632	3,512
50	4,476	3,268

shown in Table I and in the diagram (Fig 1) of duplicate counts made on two specimens, where the entire smear was counted. The variation is about a straight line and of not too large a magnitude, this is shown by the small standard deviation as compared with the value of the mean after the removal of the trend.

That it is possible to make on the same smear duplicate counts of leucocytes which check closely is shown by the results on 24 smears listed in Table II. It is also possible to make counts on duplicate smears from the same individual which check, as shown by the results of examination of 22 duplicate smears (Table III). Finally, there is as close a check with counts of the hemocytometer as one generally finds in work of this nature, for the leucocyte count varies considerably over short periods of time (Table IV). For the present at least, there is probably no need of a more accurate method of enumerating malaria parasites, for the variations that have been noted thus far as accompanying fever, etc., are many times greater than any variation due to errors in technique.

CONCLUSIONS

The main disadvantages of the Thomson method of enumerating malaria parasites in the blood, as stated by Sinton, can be easily overcome.

The method is simpler than other methods, makes possible the counting of parasites in lightly infected persons, and can be used as an accurate diagnostic method as well because of the larger amount of blood easily available for examination.

The type of pipette here described was used because it was already on the market for other purposes and was easily adapted to our needs.

The method has been used in several surveys, and duplicate counts have given results comparable to those for leucocytes (Table V).

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COOPER'S MODIFICATION OF THE ZIEHL-NEELSON STAINING METHOD AS APPLIED TO TUBERCLE BACILLI IN TISSUE*

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IN 1926 Cooper¹ reported a simple modification of the Ziehl-Neelson staining method for tubercle bacilli which, when applied to smears of sputum, demonstrates many more bacilli than the ordinary method does. The method has since been adopted and used routinely in place of the Ziehl-Neelson method by a number of laboratories. Since there appears to be no report of its application to the staining of tubercle bacilli in tissues, the following observations of a small number of duplicate sections are reported.

Carefully selected blocks of various human tuberculous tissues, fixed in Zenker's fluid and embedded in paraffin, were sectioned. Two consecutive sections from a uniformly thin ribbon were mounted from each block. After removal of the paraffin in the usual manner, one slide of each pair was stained two hours or overnight in an incubator at 38° C. with Ziehl's carbolfuchsin modified by the addition of 3 c.c. of a 10 per cent solution of sodium chloride per 100 c.c. of carbolfuchsin. These slides were then placed in an ice box for thirty minutes during which the stain was precipitated. The other slides from each pair were stained two hours or overnight at 38° C. with Ziehl's carbolfuchsin but without the addition of sodium chloride and without subsequent chilling in the ice box. From this point the treatment of both sets of slides was identical. After washing with tap water the tissues were decolorized with 5 per cent nitric acid (sp. gr. 1.4) in 95 per cent alcohol for about one minute, followed by another washing with tap water. A mixture of methylene blue and azure II was used as counterstain. This is the nuclear stain routinely employed in the Mallory phloxine-methylene blue method and is prepared from two stock solutions, one a 1 per cent aqueous solution of azure II and the other an aqueous solution of 1 per cent methylene blue and 1 per cent borax. For use mix 1 part azure II solution with 1 part borax-methylene blue solution and dilute with 18 parts of water. The diluted stain should be used immediately as it does not keep. The sections were stained for ten minutes in the methylene blue azure II solution after which the slides were washed with water and differentiated in 95 per cent alcohol containing a drop of colophonium (resin) in xylol. Dehydration, clearing, and mounting were carried out in the usual manner. There were thus available 43 duplicate sections prepared in parallel, the only difference being in the carbolfuchsin stain and its method of application.

For comparison one or more isolated tubercles containing 10 or more tubercle bacilli were selected in each section. All the tubercle bacilli in the areas selected were counted by means of oil immersion lens, No. 10 Bausch and Lomb

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binoocular eyepieces, and mechanical stage. Identical areas in each pair of sections were examined.

Structural detail of the tissue is equally distinct with both sets of slides. The blue nuclear stain does not differ appreciably in appearance from that of sections prepared by the routine phloxine-methylene blue method in which the same methylene blue-azure II mixture is used. The acid alcohol decolorization leaves the cytoplasm light red in color. The tubercle bacilli stand out as bright red rods distinctly clearer and, as Cooper noted, with fewer hairlike and beaded forms when stained with the modified carbolfuchsin. No troublesome artefacts were encountered. Further observations are as follows:

Number of cases	43
Number showing more bacilli by modified method	35
Number showing more bacilli by Ziehl-Neelson method	2
Number showing approximately equal numbers of bacilli	6
Total number of bacilli by modified method	4324
Total number of bacilli by Ziehl-Neelson method	2875

The findings of one and four-tenths times as many bacilli by the modified method corresponds to Cooper's figure of one and seven-tenths times as many in sputum smears using Loeffler's methylene blue as counterstain. Counterstains other than the methylene blue-azure II reported here are undoubtedly applicable, but brilliant green is not among these, since it is too readily removed from the tissue by alcohol. The only advantage of the blue stain suggested is that it is a color with which most pathologists are accustomed.

SUMMARY AND CONCLUSIONS

1. A method for staining tubercle bacilli in tissue is described, using Cooper's modification of carbolfuchsin.

2. The method differs but slightly from that in common use and demonstrates one and five-tenths times as many bacilli.

3. The bacilli stand out as bright red solid rods against the background of tissue in which structural detail is clearly defined.

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DETERMINATION OF P_{H^+} VALUES OF URINE*

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INTRODUCTION

IN A recent article, Pratt and Swartout¹ discuss P_{H^+} determination in routine urinalysis and make a strong plea for integrity in such work. This plea is extended to the clinician that he strike a happy medium between blind faith in routine reports and absolute contempt for reports from the most carefully controlled source.

Some time before the appearance of the paper above referred to, we engaged upon an investigation of the validity of colorimetric determination of P_{H^+} in routine urine specimens. Pratt and Swartout¹ state that there are two errors commonly met, i.e., actual P_{H^+} change on account of dilution and intrinsic alteration of indicator color by the color of a deeply tinted specimen. They set forth that they have found this last error running as high as 0.4 unit. They furthermore state that error due to dilution is less than 0.2 unit even with ratios running as high as 1:20. Myers and Muntwyler² are cited to the effect that colorimetric determination of P_{H^+} runs about 0.2 unit in error due to the determination being made at room rather than body temperature, and that this might well be corrected by subtracting this amount from the value commonly reported.

When we recall one form of the equation of Nernst as $P_{H^+} = \frac{0.7177 - 0.00074 t - V - v}{0.0001983 T}$

where t is the temperature of the solution, V the measured voltage due to potential difference between the two electrodes, v a correction factor for the potential of the calomel electrode, and T the absolute temperature, describes the condition of the quinhydrone electrode and the calomel reference electrode in terms of hydrogen ion normal, it is of course obvious that with a higher temperature and assuming it as the only permitted variable, P_{H^+} must numerically be a smaller number.

Pratt and Swartout¹ further state that inasmuch as the usual indicators for this purpose are yellow on the acid side and the intrinsic color of the specimen is a yellow, that the tendency would be to show more acid than is actually the case, while dilution on the other hand would tend to actually reduce hydrogen ion concentration or in terms of P_{H^+} to give a numerically greater value. They definitely state that the condition is one of compensating errors and that the results commonly gotten are worth fair credence. A critical study of the fundamental equation and particularly its plotted curves as met with so many times in life all the way from the population growth of a pair of fruit flies in a milk bottle, to the healing rate of a cut on the growth of a human being from the fertilized ovum to adult magnitude, causes us to suspect the strict validity of such a statement.

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Any curve of $y = e^x$ type which asymptotically approaches a limit tells us the story that variance may be slight under middle conditions but considerable as we approach one or the other limit.

The matter of "salt error" is well recognized as a source of difficulty in colorimetric measurement of P_H and is wisely noted by Myers and Muntwyler.³ However we have had occasion to watch routine procedure in various clinical laboratories, both institutional and private, over a period of years and the common procedure by better workers is to make a water dilution to compensate for specimen color and then to add the indicator and match against standard buffer tubes. The question often rose as to just what correlation existed between colorimetric and electrometric values with the result that the present work was started.

EXPERIMENTAL

This comprises the results gained by examination of one hundred routine specimens brought to a private clinical laboratory for examination. The colorimetric determinations were made with La Motte equipment, using distilled water for dilution. The electrometric measurements were made with Leeds and Northrup equipment for the quinhydrone electrode with a Clark type saturated calomel cell. All measurements were made at 25° C. both electrometric and colorimetric. The quinhydrone electrode was frequently checked against a standard buffer of $P_H = 4.63 \pm 0.01$ at 25° C. and the variance was never more than 0.02 unit. Further this standard buffer was checked by the hydrogen electrode and found to agree with the label specifications. Also, the calomel electrode was daily washed out with saturated potassium chloride solution from an outside reservoir and checked against the buffer before and after washing.

The saline diluent of Myers and Muntwyler² was disregarded for the reason that from their article it did not appear to be beyond the limit of clinical significance.

It is further set forth in the above referred to article that with salt concentrations of more than M/15 the colorimetric value is greater than the electrometric, while with smaller concentrations, the reverse is true.

The results of our investigation are given graphically in Fig. 1 and in tabular form in Table I.

The last seven colorimetric values of P_H 7.6 are stated inasmuch as they matched the buffer tube of the last indicator available at the time, and therefore equal 7.6 or some numerically greater value. The one hundred specimens were arranged in order of numerical increase of P_H as measured colorimetrically and the corresponding quinhydrone values are arranged in order of increasing deviation from their respective colorimetric values. Therefore the graph should be examined in sections or by the tabular series steps.

DISCUSSION AND EXPERIMENTAL

The first significant item is that out of one hundred specimens, but nineteen give colorimetric values in excess of electrometric values. When we recall that we dilute for a colorimetric determination, it is clear that if the equation of Nernst gives a true picture, all the quinhydrone values, made at voided concen-

tration, should represent numerically smaller P_H values. Here we have 81 per cent of our data failing to do this. Evidently we have to contend with other variables. There are at least 34 per cent of our specimens which deviate at least 0.4 P_H unit up to a maximum deviation of 2.53 P_H units. These deviations are all in the opposite direction to what would be expected according to Nernst's equation, with the only controlled variable the dilution. Two per cent of the

TABLE I

NO	P_H COLOR	P_H Q H	NO	P_H COLOR	P_H Q H
92	4.6	6.28	9	5.9	7.45
43	4.8	5.55	73	6.0	5.90
38	5.0	5.45	85	6.0	5.95
33	5.0	5.89	40	6.0	5.95
60	5.0	6.10	64	6.0	6.00
58	5.0	6.35	53	6.0	6.32
23	5.0	6.55	68	6.0	6.35
86	5.0	6.70	13	6.0	6.42
1	5.2	4.63	78	6.2	6.25
41	5.2	5.42	70	6.2	6.30
93	5.2	5.80	24	6.2	7.30
95	5.2	6.25	61	6.3	6.28
28	5.2	6.33	98	6.4	6.28
79	5.3	5.45	34	6.4	6.30
67	5.4	5.40	37	6.4	6.30
29	5.4	5.57	62	6.4	6.43
82	5.4	5.63	32	6.4	6.51
74	5.4	5.65	84	6.4	6.53
83	5.4	5.75	39	6.4	6.65
21	5.4	5.80	3	6.6	6.30
26	5.4	5.83	18	6.6	6.65
6	5.4	5.87	19	6.6	6.65
10	5.4	6.28	5	6.6	6.78
30	5.4	6.47	12	6.6	6.88
7	5.5	5.95	76	6.6	7.15
17	5.5	6.05	94	6.8	6.55
88	5.6	5.70	72	6.8	6.78
99	5.6	5.70	14	6.8	6.85
97	5.6	5.75	4	6.8	6.95
45	5.6	6.04	20	6.8	6.95
87	5.6	6.05	63	7.0	6.93
2	5.6	6.10	96	7.0	7.05
11	5.6	6.18	69	7.0	7.07
50	5.6	6.21	65	7.0	7.15
81	5.6	6.33	80	7.0	7.20
66	5.6	6.36	48	7.0	7.40
51	5.6	6.45	71	7.1	7.00
25	5.6	6.60	16	7.1	7.15
75	5.6	7.90	15	7.2	7.10
100	5.7	5.70	8	7.2	7.15
35	5.7	5.85	77	7.2	7.35
42	5.8	5.75	31	7.2	7.45
36	5.8	5.77	44	7.4	7.40
89	5.8	5.88	55	7.6*	7.14
49	5.8	6.05	90	7.6*	7.48
57	5.8	6.10	47	7.6*	8.35
59	5.8	6.40	91	7.6*	8.45
46	5.8	6.83	32	7.6*	8.58
22	5.8	7.60	27	7.6*	8.63
56	5.8	8.33	54	7.6*	8.87

* Denotes upper colorimetric limit

specimens tell more than 0.4 unit below the colorimetric values, thus showing a greater degree of acidity than measured colorimetrically. Thus we see from these figures and inspection of the curves, including the first two of the colorimetric P_{H} 7.6 values, that out of 95 reliable items a total of 41 specimens give electrometric values in excess of 0.4 unit divergent from the colorimetric ones. This leaves 54 specimens measuring electrometrically and colorimetrically within ± 0.4 unit which is certainly within the range of clinical significance.

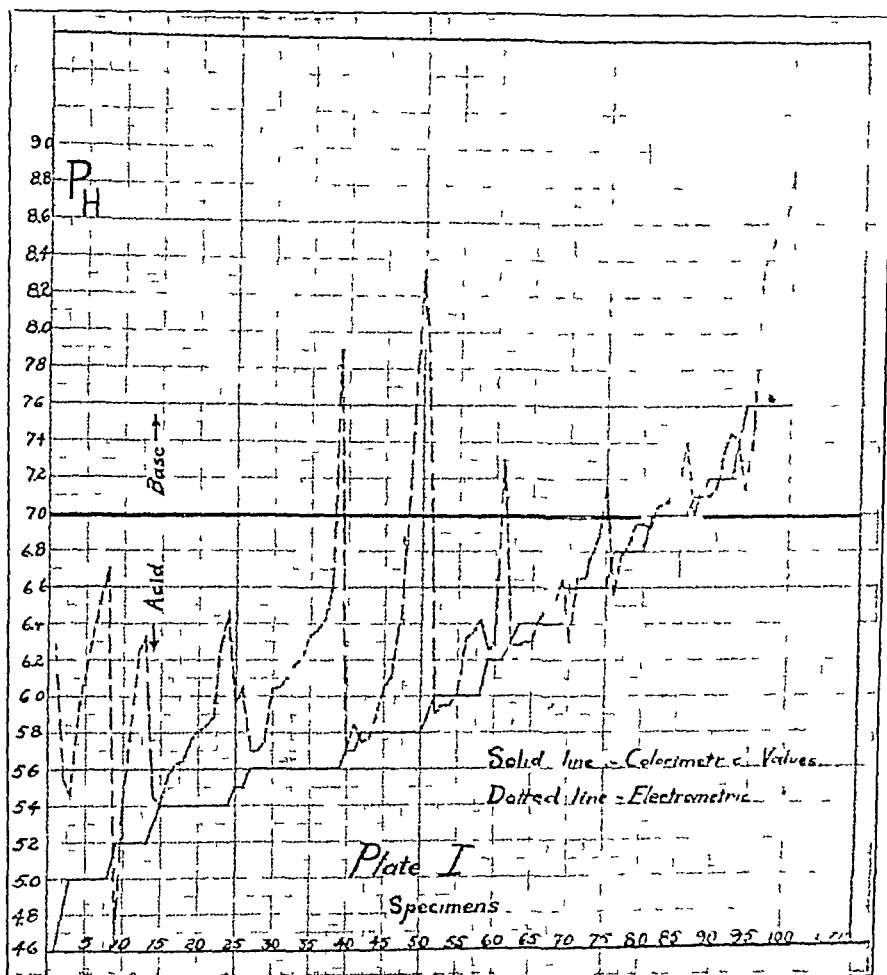


Fig 1

Again neglecting the last five of the P_{H} 7.6 specimens, we find that there are 15 specimens reading from 1.0 to 2.53 units higher numerically, or more basic than the colorimetric values. These are respectively 1.68, 1.1, 1.35, 1.55, 1.7, 1.05, 1.15, 1.07, 1.0, 2.3, 1.3, 1.65, 2.53, 1.55 and 1.1 units as picked off the graph. With respect to the heavy double P_{H} 7.0 line of neutrality drawn squarely across the graph, we see by inspection that six specimens were reported on the basis of colorimetric determination as fairly acid when they were actually basic and two of them extremely so.

In view of the foregoing, we are now forced to take stock. I. M. Kolthoff and T. Kameda¹ have recently published work which is most significant. Kolthoff has pointed out that 0.1 c.c. of 0.04 per cent methyl red when added to 10 c.c. of water of P_{H_2} 7.0, gave a color corresponding to a P_{H_2} of 5.1. He has likewise shown that in the measurements of P_{H_2} in slightly buffered solutions, reliable results are to be had only on adding an indicator of the same P_{H_2} as the unknown. Fawcett and Acree² call such indicator solutions 'adjusted' or 'isohydric.' Kolthoff and Kameda state: "As the molecular concentration of indicators in colorimetric work is of the order of 10^{-4} or smaller, the 'acid' or 'base' error will only be noticeable in solutions with extremely slight buffer action. Large errors, however, may occur when the P_{H_2} of pure water or solutions of neutral salts in water or extremely dilute solutions of acids or bases have to be measured."

Kolthoff and Kameda further refer to the work of McBain, Dubois and Hay³ and of McBain, Laing and Clark⁴ where it is stated that phenol red, *o*-cresol red, phenolphthalein, and thymol blue cannot be used for the colorimetric determination of P_{H_2} in extremely dilute sodium hydroxide solutions as the experimental figures differ by more than one or two units. The work of Kolthoff and Kameda however shows the conclusion to be unwarranted if precautions are taken against contamination by carbon dioxide, if isohydric buffer solutions are used and if the proper salt correction is used. In their conclusions they state that with isohydric phenolphthalein or thymol blue solutions the P_{H_2} of extremely dilute sodium hydroxide has been measured with an accuracy of 0.1 in P_{H_2} unit.

When the foregoing is taken coupled with the work of Myers and Muntwiler⁵ we are forced to the conclusion that with the painstaking effort of the latter workers, their variations between electrometric and colorimetric values being of considerably greater magnitude than those of Kolthoff and Kameda, there are certainly other variables playing a considerable part. Especially is this clear as we again inspect Fig. 1.

An error which has not been mentioned in any of these citations is the 'protein error' discussed by Clark⁶ and Britton.¹⁰ In view of the composition of the urine, we may safely say that we are dealing with a fluid which contains acid or base in weak concentration, various buffers as carbonates, bicarbonates and phosphates, proteins and perhaps matter in the colloidal state, certainly in those cases where albumin is found chemically and casts microscopically. It is interesting to note that in every case in Fig. 1 where an extremely wide divergence was found, albumin was present in large amounts.

At this point the question was introduced as to how P_{H_2} values by the quinhydrone electrode and the hydrogen electrode would compare. Further, the effect of buffers was clearly worthy of further study and it was clearly of importance to get all conditions into as nearly comparable a state as possible.

Therefore intensive work was engaged upon with a small number of patients having histories of albuminuria. The colorimetric determinations were made with a Hellige Klett colorimeter using standard discs, graduated tubes for dilution, standardized indicators added to the specimens in metered amounts according to the indicator used and finally plane parallel glass chambers for the specimen and color and for the plain specimen for backing the standard colors. Arti-

ficial light is used in this apparatus, thus giving constant illumination. This type of apparatus represents the finest available for colorimetric determination of P_H and is distinctly superior to the ordinary scheme of matching tubes held in the hand as done in the first hundred of Fig. 1. Colorimetric determinations were made at first on straight specimens as voided and on specimens of $2\frac{1}{2}$ c.c. diluted to 10 with distilled water. Later this was expanded as shown in Tables II and III under the headings ' P_H (ind. 1x)' to indicate a determination made with the appropriate amount of indicator and ' P_H (ind. 2x)' to indicate a determination made with twice the appropriate amount of indicator. Still later the specimens were clarified by shaking with the activated charcoal "Norit" and filtering after which the determinations were duplicated. Phosphates were de-

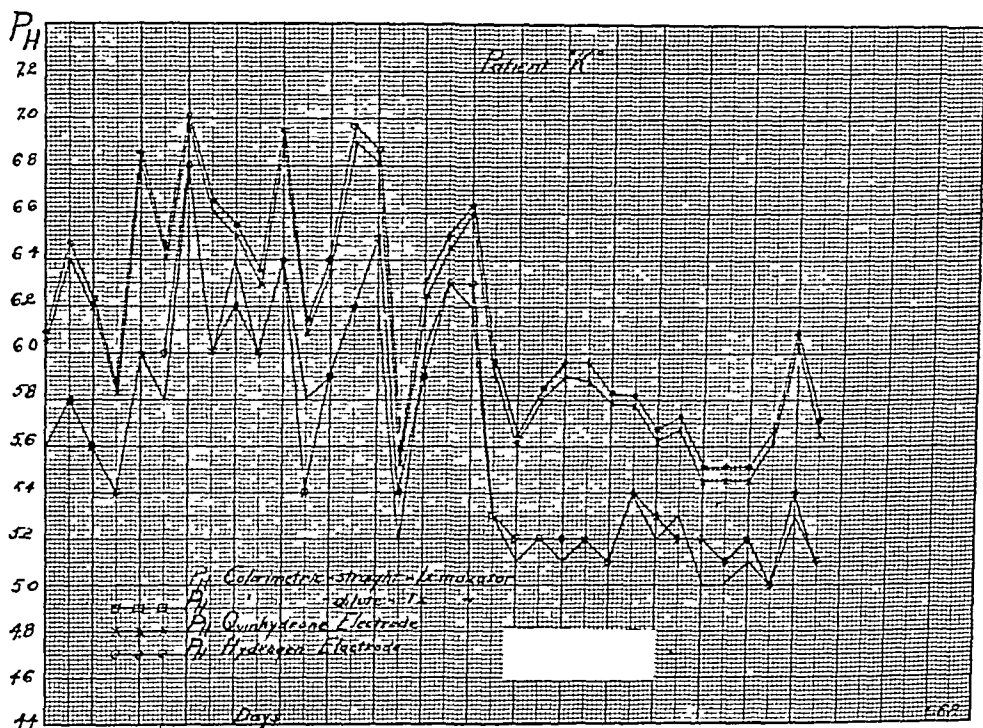


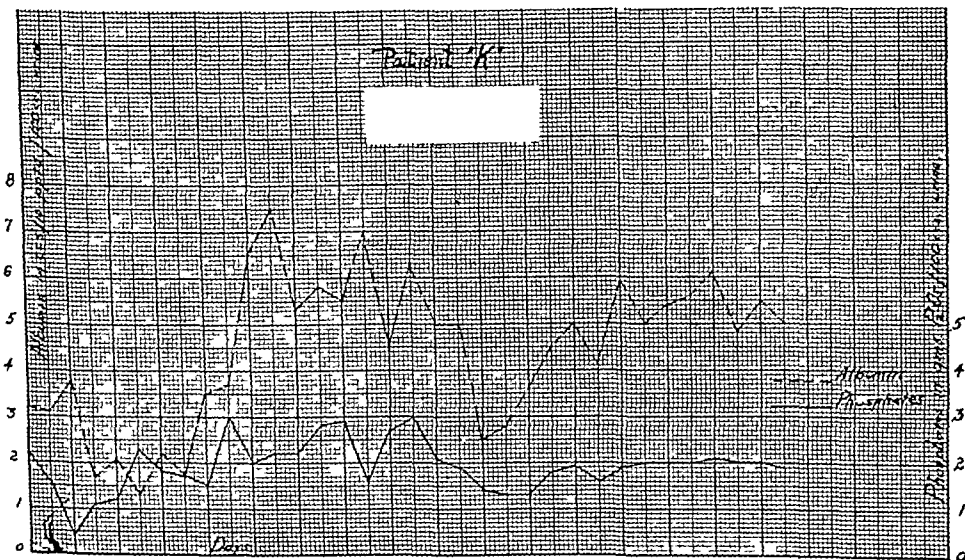
Fig. 2

termined by titration with uranium acetate using potassium ferrocyanide as an indicator and expressed in grams of phosphorus pentoxide per 100 c.c. of specimen. Albumin was determined with a calibrated centrifuge according to the method of Shevky and Stafford¹¹ and plotted on Figs. 3 and 5 for convenience in tenths of cubic centimeters of precipitated protein. Hydrogen electrode determinations were made on both clarified and unclarified specimens, the data on the unclarified specimens only being given in Tables II and III for the reason that their divergence from quinhydrone values was of exactly the same order as the clarified.

The hydrogen electrode determinations were made with Leeds and Northrup equipment, using the Type "K" potentiometer, a standard cell calibrated by the Bureau of Standards, tank hydrogen purified by bubbling through potassium

permanganate, alkaline pyrogallol and finally water to the Hildebrand hydrogen electrode which worked very nicely and came to balance well and speedily in about ten minutes. The saturated Clark type of calomel electrode was used and the 2420 c galvanometer was used. Temperature and barometric conditions were taken into account.

The first item to be observed by study of Figs 2 and 4 is that hydrogen electrode and quinhydrone electrode values are of virtually the same order. The divergence ranges from 0.02 to 0.08 P_{H^+} units, averaging very closely to 0.05 and always toward the basic side. We may therefore conclude that quinhydrone electrode values are entirely satisfactory for urines and if extreme accuracy is desired, the quinhydrone value should have added to it 0.05 units. This is entirely in accordance with the work of Cullen and Earle¹² in their work on blood plasma or serum P_{H^+} values.



Fig

From a consideration of Fig 1 it appeared that the incidence of albumin might be a large factor in the divergence between electrometric and colorimetric values of P_{H^+} . Careful consideration of Figs 2 and 3 and Figs 4 and 5 does not bear this out. The relation between albumin content and divergence between electrometric and colorimetric values is purely accidental. It is to be noted however that in none of these determinations do we have divergences of the order of the maximum of Fig 1. The reason is the vastly superior results gained by use of the Hellige Klett apparatus.

The action of buffers depends entirely on the mechanism of hydrolysis. This in turn is quantitatively affected by the equivalent buffer content. The only buffer which occurs in any great concentration in urine is phosphate. Therefore we felt that phosphate determination would give a fair measure of buffer action. Examination of Figs 2 and 3 and Figs 4 and 5 again reveals nothing more than accidental relationship. Thus we are forced to the conclusion that the urine is

under most conditions a well buffered fluid to such an extent that buffer variation is of no significance in P_{H_2} determination

Reexamination of these four illustrations however shows us clearly that variation or divergence does occur and only occasionally in the cases of these two patients did colorimetric and electrometric values approach the same value. Clarification of the specimens finally gave the answer to this. The only clarifying agent of any efficiency we found was activated charcoal 'Norit'. This worked well and speedily. It however adsorbed not only coloring matter but hydrogen ions as well, as the P_{H_2} in every case was more after clarification. It did not adsorb phosphates as the results were of exactly the same order after clarification as before and hence were not tabulated. Albumin content was essentially the same,

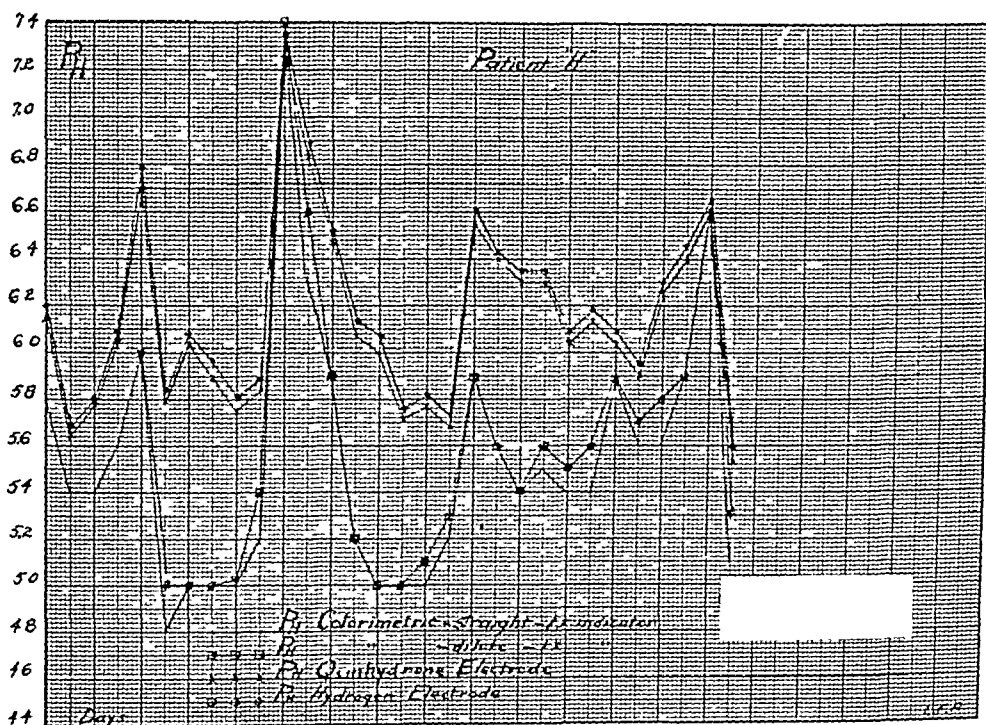


Fig 4

but in some cases if the specimen was allowed to stand a few minutes before filtering, the albumin was reduced by as much as 10 per cent. But this was in no case reduced to the minimum albumin value for either patient. Inspection of Tables II and III reveals that electrometric values after clarification are of the order of the mean value between colorimetric values with the appropriate and twice the appropriate amounts of indicator. The indicators were tried on water solutions of buffers and the agreement between electrometric and colorimetric values with the appropriate amount of indicator was perfect. Therefore we are forced to the conclusion that a part of the indicator is adsorbed by the albumin and thus made ineffective and this error could only be compensated by adding more indicator. However the fact that such values include between them the electrometric values gives us the clue to the divergence in the unclarified speci-

mens which results from the color of the specimen. The color and appearance of each specimen had been recorded and study of this record revealed the correlation. A weakly colored specimen gave a colorimetric value which was close to the electrometric. As depth of color increased, the divergence became more marked whether straight or dilute. A deeply colored specimen diverged widely. A pale specimen gave good agreement.

An item of entirely separate interest is to be seen on Figs 2 and 3 and Figs 4 and 5. In general in the case of both of these patients there was fair agreement between albumin output and hydrogen ion concentration measured electrometrically in that the higher the acidity, the lower the albumin output. Further work is in progress to determine the validity of this preliminary and incidental observation.

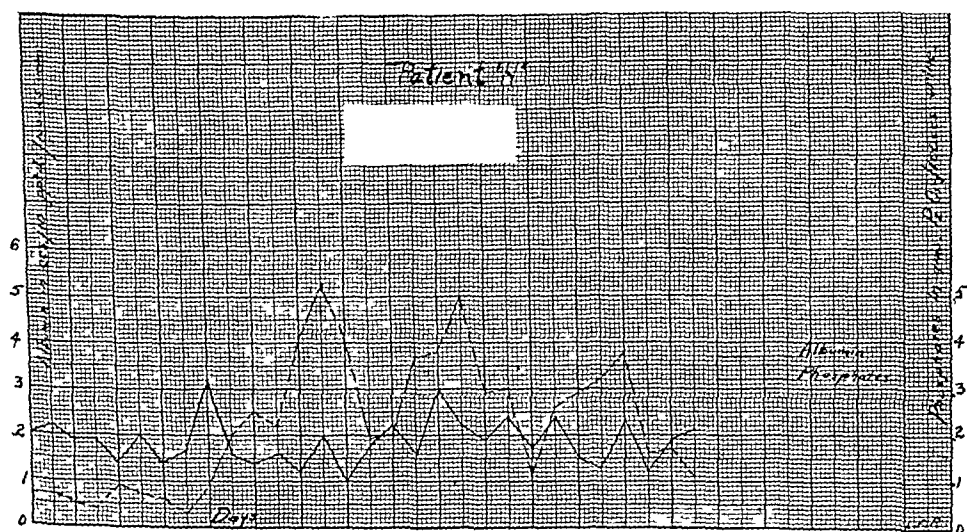


Fig 5

It is perfectly true that the quinhydrone electrode has protein errors and salt errors. Likewise it cannot be conveniently used much above P_{H_2} 8 because hydroquinone then behaves as a dibasic acid and has an effect on the hydrogen ion content of the cell. Consideration of the dissociation constants of hydroquinone as found in Britton¹⁰ shows that in unbuffered solutions of around P_{H_2} 6 the errors may be quite appreciable. Britton gives an excellent discussion of the work of La Mer and Parsons¹³ on this point. The magnitude of such errors does not rise sharply until a P_{H_2} of 8.30 is reached according to the hydrogen electrode. At this point the error is 1.24 units. We must carefully note that this error is in the direction of a false acidity or in other words under such conditions the quinhydrone electrode indicates a greater acidity than is actually the case. It is significant that the wide divergences of Figs 1, 2 and 4 are in the opposite direction or toward the basic side. With respect to the protein error, Kolthoff¹⁴ has shown it to be 1.24 units with casein and sodium hydroxide while with blood serum and buffers the error is no more than -0.18 unit.

It must not be forgotten that indicators are usually acids made up in base,

and they certainly have the potentiality of neutralizing a part of the base of a specimen, thus giving an erroneous color.

SUMMARY

1 A comparison of P_H values in urine determined in accordance with average good clinical laboratory procedure, electrometrically and colorimetrically shows a trifle more than a one to one chance of the colorimetric value being within a limit of ± 0.4 unit of the electrometric true value.

2 At least 7 per cent of a hundred routine specimens of urine are actually far more basic than shown by colorimetric determination by numerical magnitudes of scientific and perhaps clinical interest. In the case of grave illness this percentage jumps to over twenty six.

3 These divergences arise from the intrinsic color of the specimen.

4 Clarification experiments have shown that even removal of coloring matter still exposes fundamental weakness of colorimetry with its "estimations" as compared with electrometric determinations.

5 The direction of divergence between electrometric determinations on undiluted specimens and colorimetric determinations on both diluted and straight specimens is largely an exact opposite of what might be expected from the equation of Nernst, showing conclusively that the dilution error is neither the only nor the largest error encountered.

6 If P_H determinations are to be made on urines they should be done with the quinhydrone electrode which is simple, of no greater cost than a good colorimetric outfit, capable of faster handling than colorimetric equipment of the best kind and very easily cared for.

7 It appears that albumin output is reduced in general as acidity of the urine rises in at least some cases. Further work is in progress on this point.

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PHOSPHORUS METABOLISM

III ON THE DETERMINATION OF PHOSPHORUS IN URINE*

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THE method by which phosphomolybdate is reduced with stannous chloride to give a blue color recently adapted to the determination of phosphate in blood,¹ can be readily applied in simplified form to urine. There are no interfering substances in sufficient concentration. The method is accurate and rapid. Very little urine is required.

The following is the outline for the determination of inorganic phosphate (If total phosphorus is desired, oxidation with sulphuric acid and hydrogen peroxide as for blood must be done.)

SPECIAL REAGENTS REQUIRED

1 Molybdate Sulphuric Acid Mixture. Mix 50 cc of 7.5 per cent sodium molybdate $Na_2MoO_4 \cdot 2H_2O$, c p (P free), 25 cc of water, and 25 cc 10 normal sulphuric acid. (The same volume of 12.8 per cent ammonium molybdate, $(NH_4)_6Mo_7 \cdot 4H_2O$, may replace the sodium molybdate solution.)

2 Stannous Chloride Solutions (stock and dilute solutions as prescribed by Kuttner and Cohen²). Dissolve 10 grams c p stannous chloride in 25 cc concentrated hydrochloric acid. Store in a brown glass stoppered bottle. Dilute 1 cc of the above stock solution to 200 cc with water. A new dilution is made about every 5 days unless a turbidity should develop sooner.

3 Standard Phosphate Solution.—Dissolve 0.4389 gram pure dry monopotassium phosphate in water enough to make 1000 cc. 10 cc = 1 mg P. Dilute 10 cc of the stock solution to 100 cc. 1 cc = 0.01 mg P. Preserve by acidifying with sulphuric acid.

PROCEDURE

Dilute 1 cc of urine to 100 cc. Of this diluted urine transfer 1 cc and 2 cc to test tubes accurately graduated at 10 cc. Transfer 2 cc (0.02 mg P) of standard phosphate solution to a similar test tube. Add to each tube water to make about 6 cc, then 2 cc of molybdate sulphuric acid mixture, 1 cc of dilute stannous chloride solution, and water to the mark. The addition of stannous

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If test tubes graduated at 20 cc or 50 cc volumetric flasks may be used with proportionately more of urine and reagents.

nous chloride, water to the mark, and final mixing should be done without delay. After 1 minute compare in the colorimeter the tube which comes nearer in color to the standard. If the color does not come within 30 per cent of the standard, the determination must be repeated with a corrected amount of urine.

Calculation

$$\frac{\text{Reading of standard}}{\text{Reading of urine}} \times 0.02 \times \frac{100}{\text{cc dil urine taken}} = \text{mg P in 1 cc urine}$$

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A MICRO METHOD FOR BLOOD UREA NITROGEN*

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WITH the growth of chemical analysis of blood in routine hospital laboratories, a demand has arisen for micro methods of analysis. These methods should not replace the usual routine methods but should be used only as an emergency laboratory procedure in connection with patients of difficult venipuncture. To make a special set of reagents for the occasional use of micro methods is time-consuming and expensive. With this in mind the following method was developed, using the reagents of Kall's¹ Urea Nitrogen Method and making one new solution for precipitation of proteins.

Protein is precipitated from blood by means of tungstic acid. To the filtrate is added urease and phosphate buffer. It is then incubated for ten minutes at 50° C. The resultant solution is nesslerized and compared colorimetrically with a similarly treated standard urea solution.

The only special apparatus necessary is two test tubes, each graduated at nine and ten cubic centimeters.

Solutions—Tungstic acid. To 80 c.c. of water add 16 c.c. of N/12 sulphuric acid volumetric solution. Mix and add 2 c.c. of 10 per cent sodium tungstate solution. The sodium tungstate is of the "Special" grade. Urease solution. 15 gm. of Jack bean meal and 5 gm. of Permutit are placed in an Erlenmeyer flask. A mixture of 16 c.c. of alcohol and 84 c.c. of water is added. This is shaken continuously for ten minutes, then allowed to stand 18 hours in a refrigerator, after which it is filtered and kept in the refrigerator. Phosphate buffer. Dissolve 14 gm. of sodium pyrophosphate and 2 gm. of metaphosphoric acid in water and dilute to 250 c.c. Urea stock solution. Dissolve 0.1286 gm. of urea in water and dilute to 200 c.c. Urea standard solution. Dilute 5 c.c. of the stock

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urea solution to 100 c c with distilled water 5 c c is equivalent to 0.075 mg of urea nitrogen Nessler's solution

Method Pipette 9.8 c c of the tungstic acid solution into a 15 c c centrifuge tube Cleanse the end of the patient's finger with alcohol and when the skin is dry, prick with a blood lancet Using a 0.2 c c serologic pipette (that delivers to the tip) collect 0.2 c c of blood Deliver it into the tungstic acid solution, rinsing the pipette with the same Stopper the tube and mix thoroughly Allow to stand a few minutes, then centrifuge

Transfer 4 c c of the supernatant fluid to a small test tube Into a similar test tube pipette 1 c c of urea nitrogen standard and 3 c c of water To each tube add 3 drops of urease and 3 drops of the buffer solution Incubate both tubes in a water-bath at 50° C for 10 minutes At the end of this time transfer the contents of each tube to a tube graduated at 9 and 10 c c, rinsing the first tube with distilled water and diluting to the 9 c c graduation Add Nessler's solution to the 10 c c graduation and compare colorimetrically in a Dubosque type of colorimeter

Calculation If the unknown is set at 15 mm then the reading of the standard multiplied by $1\frac{1}{4}$ or 1.25 gives milligrams of urea nitrogen per 100 c c of blood

The following results were obtained, using Karr's method on venous blood and this micro method on blood obtained from a finger prick

KARR'S METHOD	KELLER'S METHOD
RESULTS EXPRESSED IN MG PER 100 C C OF BLOOD	
10.0	10.0
12.3	12.1
14.8	14.6
15.0	15.0
13.5	13.3
11.8	11.6
12.0	12.0

CONCLUSION

A micro method for blood urea nitrogen is offered which checks with methods using larger quantities of blood

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THE DETERMINATION OF CALCIUM AND PHOSPHORUS IN SALIVA*†

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INTEREST in the relationship of calcium and phosphorus in saliva to dental decay necessitated an investigation of the various procedures for the estimation of these substances

CALCIUM

The most adaptable methods appeared to be either the ashing process as recently described by Leonard* for saliva calcium or the precipitation of calcium after preliminary treatment with trichloroacetic acid In carrying out the former technic we found a fine pellicle of calcium oxalate on the surface of the supernatant fluid after washing the precipitate of calcium oxalate with NH_4OH (The same effect is obtained when calcium is determined in an HCl solution of ashed bone or teeth, and in other inorganic solutions) The surface pellicle was lost when the supernatant fluid was decanted, resulting in a somewhat lower calcium recovery, as we shall demonstrate presently During the process of ashing all protein or other organic material was removed, so that when the ash was dissolved in HCl , the resulting solution had no colloidal components We felt that the calcium oxalate particles need some binder in order that they may form an adhering mass To effect this we precipitated the calcium in the HCl solution obtained from the ash with saliva saturated with ammonium oxalate The

TABLE I
RECOVERY OF CALCIUM BY DIFFERENT METHODS

SAMPLE NUMBER	MG CA IN 100 CC OF CA SOLUTION									
	1	2	3	4	5	6	7	8	9	10
A Precipitation with ammonium oxalate	59	57	57	59	53	57	56	60	59	54
B Precipitation with ammonium oxalate saliva	60	61	59	58	60	58	59	58	63	61
C Precipitation with ammonium oxalate albumin	60	62	60	60	62	60	62	60		
D Precipitation with ammonium oxalate and addition of acetone before NH_4OH washing	60	60	61	60	60	60	60	60	60	60

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latter was prepared by adding to 10 c.c. of freshly collected saliva one gram of finely ground ammonium oxalate. This mixture was shaken for two hours and centrifuged. The decanted supernatant fluid is the ammonium oxalate-saliva used. The results are often higher, and one is spared the anxiety that the calcium oxalate precipitated in one step is being lost in the next. The comparison is brought out in Table I (A and B) and in Table II.

The calcium solution mentioned in the table contained 0.1498 gm CaCO_3 , 0.5265 gm KH_2PO_4 and 0.0709 gm $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ in 1000 c.c. of a 0.4 N solution of HCl. There were therefore, 6 mg Ca, 12 mg P and 0.7 mg Mg in 100 c.c., which are average figures for these constituents in saliva.

TABLE II
DETERMINATION OF CALCIUM BY DIFFERENT METHODS

SAMPLE NUMBER	MG CA PER 100 C.C. OF SALIVA							
	1	2	3	4	5	6	7	8
Precipitation with ammonium oxalate	43	40	45	42	46	44	57	43
Precipitation with ammonium oxalate saliva	54	41	47	42	46	44	60	46

TABLE III
DETERMINATION OF CALCIUM BY DIFFERENT METHODS

DETERMINATION OF CALCIUM BY DIFFERENCE											
SAMPLE NUMBER	MG CA IN 100 CC OF SALIVA										
	1F	3F	1L	3L	40	41	42	44	39	45	46
Precipitation with ammonium oxalate saliva	48	50	44	45	54	80	87	62	59	74	59
Precipitation with ammonium oxalate albumin solution	50	49	43	45	55	79	93	67	57	73	56

To avoid the collection of saliva, an ammonium oxalate-albumin solution was substituted for the ammonium oxalate saliva. The albumin solution was prepared as follows. To 100 c.c. of a fresh albumin solution was added one gram of finely ground ammonium oxalate and the mixture shaken for two hours. The supernatant fluid obtained after centrifugation was successfully used for precipitating calcium as calcium oxalate. This is demonstrated in Table I (C) and Table III.

It was later found that the addition of acetone to the calcium oxalate precipitate before washing it with ammonium hydroxide prevented pellicle formation by reducing the surface tension. That this procedure yields accurate results is brought out in Table I (D) which gives calcium recovery values for the calcium solution previously used.

The description of the method is finally recommended follows: 10 c.c. of saliva in a 40 c.c. platinum crucible are evaporated to dryness on a steam bath.

The residue is carefully charred over a low gas flame and then ignited in a furnace at 600 to 650° C for one hour. After cooling, the ash is dissolved in 3 cc HCl (0.4 N) using 1 cc at a time. Each portion is successively transferred to a 10 cc volumetric flask with another pipette. The crucible is then washed three times with approximately 2 cc of water for each washing and transferred in the same way as was the HCl. Water is added to make 10 cc.

Five cubic centimeters of this solution in a 15 cc conical centrifuge tube are adjusted to P_H 5.9 using 0.1 cc of 0.04 per cent bromocresol purple as the indicator.¹ The adjustments are made with 5, 0.25, and 0.05 N NH_4OH , and 1.01 and 0.01 N HCl until a lavender-gray color is obtained. Three cubic centimeters of 4 per cent ammonium oxalate solution having a P_H of 5.9 are added, allowed to stand for one hour, and centrifuged for ten minutes at about 1300 r.p.m. The supernatant fluid is carefully decanted. The tube is drained on filter paper for five minutes and the rim wiped. The precipitate is thoroughly mixed with 0.5 cc acetone, then 3 cc NH_4OH (2 cc concentrated NH_4OH to 98 cc H_2O) are added in such manner that the whole surface of the tube is washed thoroughly. The mixture is gently agitated. After centrifuging for five minutes at 1300 r.p.m., the supernatant fluid is poured off. Again 0.5 cc acetone is added followed by 3 cc NH_4OH carefully washing the sides of the tube *without* disturbing the precipitate, and the centrifuging and draining described above repeated. The calcium oxalate in the centrifuge tube is mixed well with a few drops of N H_2SO_4 and then 2 cc are added washing the tube at the same time. The titration is carried out with 0.005 N $KMnO_4$.* The following blank is subtracted from the titration figure: 15 cc HCl (0.4 N) + 35 cc H_2O . The P_H is adjusted as in the determination, 3 cc ammonium oxalate solution are added and all the steps in the above description carried out.

The mg Ca per 100 cc of saliva may be obtained from the equation

$$\frac{\text{cc standard sodium oxalate solution}}{\text{cc } KMnO_4 \text{ used as titration equivalent}} \times 0.02 \times 40/2 \times R \times 20 = X$$

R = number of cc $KMnO_4$ used in titrating the sample minus blank

40/2 = equivalent weight of calcium

DETERMINATION OF CALCIUM WITHOUT ASHING

While experimenting with the ashing method for the determination of calcium, we tested the possibility of estimating calcium in saliva without previous ignition. The following technique was evolved.

To 8 cc of saliva in a 15 cc conical centrifuge tube are added 2 cc of CCl_3COOH (30 per cent by volume). The mixture is stirred with a fine rod, stoppered and allowed to stand for ten minutes. After centrifuging for five minutes at 1300 r.p.m. any pellicle that may form is removed with a fine capillary pipette. The supernatant fluid is decanted into another 15 cc conical centrifuge tube. (If measurements are not made immediately, the tube should be tightly stoppered.)

Five cubic centimeters in a 15 cc conical centrifuge tube are adjusted as in

*The 0.005 N $KMnO_4$ is prepared by diluting a stock 0.1 N solution. The diluted solution should be standardized with each set of determinations against 0.02 N sodium oxalate (0.134 gm sodium oxalate is dissolved in 100 cc H_2O containing 0.5 cc H_2SO_4 of specific gravity 1.84).

the ashing method to $P_{H\ 5.9}$, 30 c c of a 4 per cent ammonium oxalate solution, previously adjusted to $P_{H\ 5.9}$ with dilute oxalic acid or dilute NH_4OH , are added. The mixture is allowed to stand for two hours and centrifuged for ten minutes at about 1300 r.p.m. The procedure from this point parallels that in the ashing method except for the blank. For this purpose, the remaining portion of the supernatant fluid is treated exactly as outlined above, omitting the addition of ammonium oxalate.

The calculation for calcium determined by direct precipitation is the same as that for ashing technique. It must not be overlooked, however, that the aliquot used in the former is equivalent to only 4 c c of saliva. (In the formula, 25 is substituted for 20.)

It is interesting to note how closely the results obtained by the two methods check each other (Tables IV and V).

TABLE IV
DETERMINATION OF CALCIUM IN ASHED AND UNASHED SALIVA

SAMPLE NUMBER	MG CA PER 100 C C												
	4c	4d	5c	5d	7a	8a	8b	8c	8d	9a	9b	9c	9d
Determination on ashed saliva	4.3	4.4	4.9	5.1	4.6	4.7	5.6	5.5	5.1	4.4	4.7	4.5	4.7
Determination on unashed saliva	4.2	4.3	4.7	5.0	4.8	4.7	5.7	5.4	5.0	4.3	4.7	4.5	4.7

TABLE V
DETERMINATION OF CALCIUM IN ASHED AND UNASHED SALIVA

SAMPLE NUMBER	MG CA PER 100 C C													
	10b	11a	11b	12a	12b	14a	14b	15a	16a	17a	17b	18a	19a	19b
Determination on ashed saliva	3.9	5.8	6.1	4.7	4.9	4.7	4.9	4.5	4.1	4.7	4.9	5.3	4.7	4.0
Determination on unashed saliva	3.8	5.9	6.3	4.6	4.7	4.7	5.0	4.3	3.6	4.7	4.6	5.2	4.4	4.4

The figures obtained by precipitation of calcium from the supernatant fluid reproduce those obtained by the ashing technique so closely that the results appear as though they were duplicate determinations carried out by the same method. Besides greatly curtailing the time necessary for each analysis, direct precipitation of calcium eliminates the need for a muffle furnace and platinum crucibles. It is therefore, to be preferred to other methods. Especially is this true when a large number of samples have to be handled.

PHOSPHORUS

There was a choice of two methods, that of Fiske and Subbarow which is short and simple, and that of Tisdall which although more difficult, is applicable when only very small quantities of material are available. Both of these methods gave comparable results when applied to a HCl solution of ashed saliva (Tables

VI and VII, compare same sample numbers) However, when the analyses were carried out by the Fiske and Subbarow method, the results obtained on the ash solution differed markedly from those obtained on the supernatant fluid after trichloroacetic acid treatment (Table VI) On the other hand, by using the Tisdall procedure on these solutions, close checks were obtained (Table VII and Table VIII)

TABLE VI
DETERMINATION OF PHOSPHORUS BY FISKE AND SUBBAROW METHOD

SAMPLE NUMBER	MG PER 100 CC SALIVA			
	1a	2a	4a	5a
Ash solution	10.9	9.6	9.4	10.6
CCl ₃ COOH supernatant fluid	8.3	7.2	6.3	6.6

TABLE VII
DETERMINATION OF PHOSPHORUS IN UNASHED AND ASHED SALIVA

SAMPLE NUMBER	MG PER 100 CC (TISDALL METHOD)										
	1a	1b	1c	1d	2a	3a	3f	3k	5c	5d	6a
Unashed saliva	10.8	11.2	10.2	10.4	10.4	11.8	13.9	17.2	10.1	8.9	11.0
Ashed saliva	10.6	10.8	10.0	10.4	9.9	11.1	13.5	16.8	9.7	9.0	10.9

TABLE VIII
DETERMINATION OF PHOSPHORUS IN UNASHED AND ASHED SALIVA

SAMPLE NUMBER	MG PER 100 CC (TISDALL METHOD)									
	10b	11a	11b	12a	12b	15a	16w	17a	18a	19b
Unashed saliva	9.1	8.8	9.4	8.7	8.8	12.1	14.1	10.5	10.7	13.7
Ashed saliva	8.8	8.9	9.3	8.8	9.3	11.4	13.9	10.6	10.5	13.4

In applying the Tisdall technique to the supernatant fluid after trichloroacetic acid precipitation 0.5 cc is used, and the process is continued as directed in the original description⁴

SUMMARY

It has been shown that calcium and phosphorus can be determined on unashed saliva. The results check very closely those obtained on a hydrochloric acid solution of saliva ash. The method recommended for the determination of calcium in ashed saliva is probably applicable to the determination of calcium in other biologic material.

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AN OPERATING TABLE FOR SMALL ANIMALS*

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FIG 1 illustrates a table† that has been used in the pharmacology laboratory for the past three years. It will hold rats securely in place while making intravenous injections or during major surgical operations under anesthesia. The method of tying the animal in place as well as releasing it at the conclusion of an experiment provides both for speed of manipulation and certainty of action.

The top of the table is 18.5 by 32 by 2 cm. and stands 65 cm. high. In each corner of the top there is a spring clip for holding the leg strings at whatever tension desired. Each clip is constructed as follows: A brass sleeve 20 mm. long, O. D. 11 mm. and a bore of 7.25 mm. is forced into place through a hole in each corner of the board. Through this brass cylinder passes a 7 mm. brass rod, 45 mm. long. In the upper end of the rod is cut a notch and on the end just over the notch there is a 10 mm. cap (See Fig 1-E, a). The lower end of this movable rod is likewise fitted with a 10 mm. cap (E, f) and between the cap and the surface of the board is a spring (E, e). This spring forces the upper end of the movable rod into the brass cylinder until the cap fits tightly against the end of the cylinder and if a cord is drawn into the notch it is held firmly *in situ* upon releasing the thumb pressure upon the lower cap. (See Fig 1-B, s and v). The spring is similar to those used in door locks and is made of spring wire, No. 16 or 17 B and S gage.

Fig 1 B illustrates the manner of operating the string holder just described. A double cord (t) of suitable thickness is looped around the leg of the animal (Fig 1 B, v) drawn into position and passed through the notch of the string holder (B, b) and the thumb removed so that the spring draws the rod down upon the cord (Fig 1-B, u, v). After first setting all four strings attached each to one leg respectively of the animal and thereby approximating its desired position, final adjustments are made by simply pressing the string holder upwards and adjusting the leg strings. Finally wrap the cord once around the notch (Fig 1 B, s) and let the string holder seat itself by removing the thumb pressure.

If the springs are strong enough this precaution of winding the string once around the movable post is not necessary. So it is well worth the trouble to make good strong springs of phosphorus bronze spring wire. The notch will then grip the cord tight enough to hold it where placed even when pulled at vigorously. Animals, therefore stretched out properly upon the operating board, even though struggling vigorously can not draw the leg strings through the gripping notch of the string holder.

When releasing an animal, the thumb is placed upon the lower cap and by pressure the piston is thrust upwards and the notch releases the string. Then

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†The mechanical details that are responsible for the appearance and working efficiency of this animal board is due to the excellent workmanship of Mr. Willie H. Hume, demonstrator and clerk in the Department of Pharmacology, School of Medicine of the University of Maryland. Received for publication December 27, 1931.

by means of a pair of tweezers the loop on the leg is released, slipped off, and the animal removed. The strings are left in place threaded through the notch for future use.

A second type of animal board is much more simple in construction. The string clips of this board are illustrated in the insert, Fig. 1-A. In many respects this board is more convenient for holding small animals like rats and mice. It presents flat surfaces with no obstructing supports; hence, when no stands or other obstructions upon the laboratory desk need be straddled, this board is very convenient.

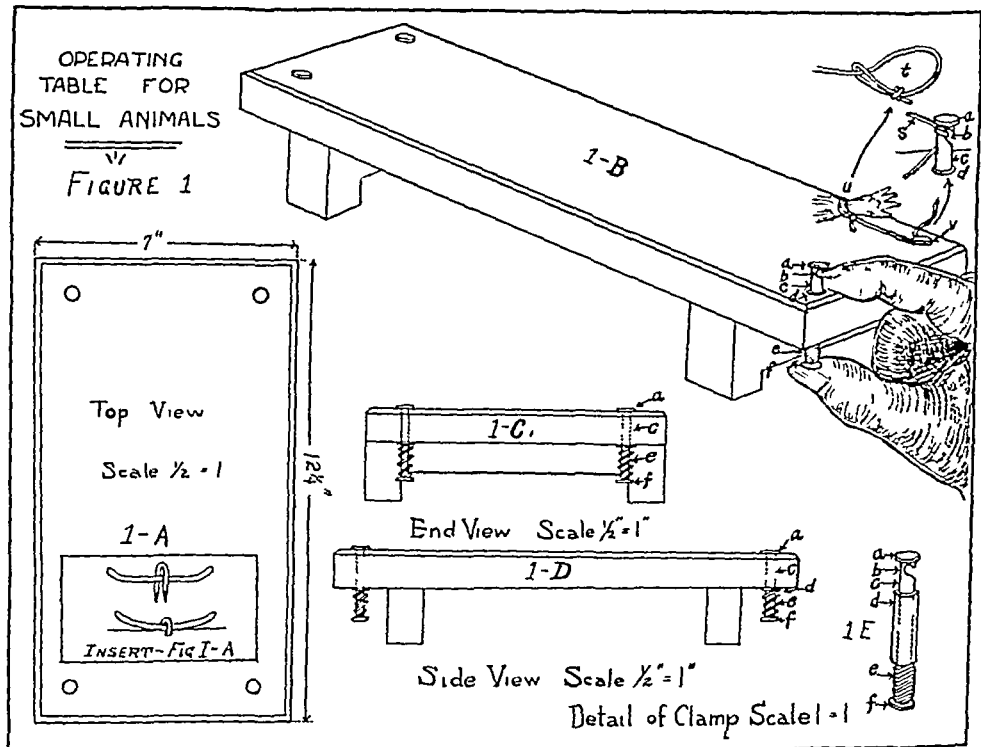


FIG. 1

It is made of good quality spruce or white pine, 12 to 15 mm thick. For rats the top is 15 by 30 cm. For mice and frogs, the top may be reduced to 14 by 20 cm. In each corner is fastened a piece of phosphorus bronze spring wire No. 14, (B and S gage) about 35 mm long. The end of this wire is rounded. Seven millimeters from each end the wire is bent so that the straight portion of the original wire is resting upon a plane surface; the upturned ends will subtend an angle of about 10 to 15° with the plane surface or with the straight portion of wire projected along this surface. (See Insert Fig. 1-A.)

One wire each is then fastened in each respective corner of the board by means of a staple, so that the bent ends of each wire project upwards. Upon driving the staple home it grips the middle of the wire and not only holds it in place, but also increases the angle between the surface of the board and the upturned end of the wire.

The leg strings described in connection with Fig 1-B, *t*, are used in the same manner, except that instead of passing the string through the notch (Fig 1 B, *b*) the string is forced between the wire and the board. If it is necessary to make doubly certain that the string will not slip, then it is wound once around the fastener, thereby making use of both ends of the wire to grip and hold the leg string in place. When made properly these string-grips are very efficient, they not only hold the string firmly but admit of quick adjustments, rapid fixation, and equally rapid release.

THE KAHN PRECIPITATION TEST THE USE OF A SINGLE TUBE WITH THE OPTIMUM PROPORTION OF SERUM AND ANTIGEN*

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KAHN'S original precipitation test¹ consisted of a single mixture of serum and antigen. Subsequent modification² has resulted in the routine use of three different antigen serum proportions. The reasons for making the test with different amounts of antigen, as stated by the author,³ are twofold: (1) different serums and different stages of syphilis have different optimums for precipitation, (2) the three tube test gives, to some extent, at least, a quantitative result. Kahn states⁴ that five or more tubes would be better, but three were selected for practical reasons. Any quantitative element which might be elicited is, however, nullified by the practice of reporting a single result, obtained by averaging the readings of the three tubes. Furthermore, Kahn⁵ has proposed an additional quantitative test which more closely approximates a titration of the serum on the basis of "reacting units."

Levine⁶ has recently proposed the omission of the 3:1 serum antigen mixture, and, on the basis of a two tube test reading, has reported results, from 500 positive serums, nearly identical with the three tube readings. He points out at least one definite advantage, namely, the elimination of a large number of doubtful (1+) reactions.

A quantitative test may have a place in the clinical or hospital laboratory where close contact with the patient is possible, and where serologic observations are desirable during treatment, but it has no place in a public health laboratory whose chief function is the detection of syphilitic infection. Particularly is this true if a complement fixation test is included in conjunction with the precipitation reaction, one serving as a check upon the other. Under these conditions, a one tube precipitation test utilizing that ratio of serum to antigen which gives the most specific results would be sufficient.

It is obvious that the proportion which yields the highest degree of specificity lies somewhere between 3:1 and 12:1. The former usually gives a negative result except with strongly positive serums while the latter yields in our ex-

*From the Laboratories of the Alabama State Board of Health.
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perience, about 5 per cent more positive results than does the Kolmer complement fixation technic, indicating that a proportion of serum to antigen much greater than this would be too sensitive.

In order to determine the comparative results obtained by the use of a single mixture of antigen and serum or a combination of two different mixtures, the results in each of the three tubes in 1000 positive serums have been recorded separately. These have been compared with each other and with the results of the three tube test and the two tube reading proposed by Levine. There were 209 serums which gave different degrees of precipitation in the three tubes. The other 791 serums yielded the same amount of precipitation in each tube. The differences are summarized in Table I.

TABLE I
RESULTS WITH 209 SERUMS GIVING DIFFERENT DEGREES OF PRECIPITATION WITH VARIOUS
SERUM ANTIGEN MIXTURES

THREE TUBE RESULT	TWO TUBE RESULT (6:1 AND 12:1)	ONE TUBE RESULT (12:1)	NO. OF SERUMS
1+	1+	2+	2
1+	2+	2+	54
1+	2+	3+	22
2+	3+	3+	49
2+	3+	4+	15
2+	4+	4+	1
3+	4+	4+	66
Total Difference—1 and 3 tube—209—20.9%			
Total Difference—2 and 3 tube—207—20.7%			
Total Difference—1 and 2 tube—39—3.9%			

There were 72 specimens which yielded a greater precipitation in the 12:1 ratio than in the 6:1 tube. Seventeen were negative and 29 were 1- in the 6:1 proportion but positive in the 12:1 tube. Ten (1 per cent) were 1+ in the 12:1 proportion, while 88 (88 per cent) gave a doubtful result in the 3 tube test. These results indicate that the optimum ratio of serum to antigen is more than 6:1, and probably in the neighborhood of 12:1.

There were no qualitative differences in this series. The greatest quantitative difference obtained between the two and three tube results was a change of 1+ in the result, from 1+ to 2+, 2+ to 3+, or 3+ to 4+. These results are in accord with those reported by Levine.⁶ Furthermore, the 12:1 result was stronger than the three tube average by a 2+ amount in only 38 specimens.

There were only 39 serums which yielded a greater precipitate in the 12:1 ratio alone than in the average of the 6:1 and 12:1 tubes, this was never a difference of more than 1+. When the results of the two tube test and the one tube (12:1) test are compared with the standard test, the differences are immaterial, 209 in the one tube and 207 in the average of the two tubes.

Such differences as those which have appeared in this series of tests are entirely within the limits of error of the technic. In a series of over 500 syphilitic serums which have been examined independently by each of our eight branch laboratories, all using the same antigen prepared by the Central Laboratory, differences as great as those obtained in this series of tests, were reported. In the case of weakly positive serums, particularly, in the hands of different

serologists, even qualitative differences, as well as those merely of degree, may be obtained, when the three antigen proportions are used. This indicates that the personal element has not been eliminated as a factor in this test, and, further, that the use of the optimum ratio of serum and antigen for specific precipitation is preferable to the practice of averaging the results of several mixtures.

The following conclusions, therefore, seem justified.

1 The optimum mixture of serum and antigen for specific precipitation is approximately 12 : 1. This proportion apparently holds for the great majority, at least, of all specimens.

2 The use of this optimum ratio alone or of an additional one (e. g. 6 : 1) also, does not yield falsely positive results. On the other hand, the doubtful (1+) results are reduced from 8.8 per cent (in this series) to 1 per cent.

3 It seems apparent that the use of one tube using a 12 : 1 proportion of serum and antigen, in conjunction with a dependable complement fixation reaction, constitutes a valuable qualitative test for syphilis, to the reliability of which nothing is added by the use of additional serum antigen proportions in the precipitation test.

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AN APPARATUS FOR QUICKLY MEASURING THE SPECIFIC GRAVITY OF BODY FLUIDS*

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INTEREST was aroused in the apparent practicability and value of such a method by Barbour and Hamilton's description of their method in 1924¹. Essentially it consists of measuring the falling time of a small drop of blood or other watery liquid in a tube of immiscible liquid of known specific gravity, as chloroform, benzol, or, as recommended by Barbour and Hamilton brombenzene—xylene mixture. The great advantage of the latter mixture against the earlier ones tried, as chloroform-benzene xylene or gasoline, is their relatively great stability of gravity even after considerable exposure to evaporation.

Burette-like tubes measuring 16 by 400 mm. are used, the falling time between two marks 30 cm. apart being measured. The liquid is measured and placed in the gravity mixtures by means of a simple straight pipette, the liquid discharging by gravity, and the quantity being measured between marks. Each apparatus carries three tubes of the mixtures having gravities of 1.010, 1.020, and 1.040 which covers the maximum and minimum ranges of blood and serum (Fig. 1). By more than five years' study of the method in use by students, it is clear that in comparison with other methods for evaluating blood conditions, as red cell count, and hemoglobin by various methods it stands first from every standpoint. And calculations of red cells, hemoglobin, etc., from comparison tables prepared from actual findings on bloods of known composition, show unexpected accuracy.

The method is not automatic or proof against misuse, but it is comparatively simple and rapid, and accurate within the limits of application recommended. Like any other method it requires reasonable attention to keep in good order, such as proper washing of the pipettes, and occasional testing of the gravity of the mixtures with the standard gravity solutions.

The compact form shown in the figures includes a split second timer, which much experience has proved to be at least as accurate as the average moderate priced Swiss laboratory stop watch.

The one handed pipette controller likewise has proved very efficient and satisfactory.

In making a test but a moment is required to insert a pipette into the controller and immerse it with concentrated sodium citrate solution.

The ear or finger is then punctured in the usual manner from which time the test is completed and the thermometer and table read in not more than one minute (Table I). Since the pipette is calibrated between two marks, and from the lower mark to the point, a second or control test can be made from the one filling.

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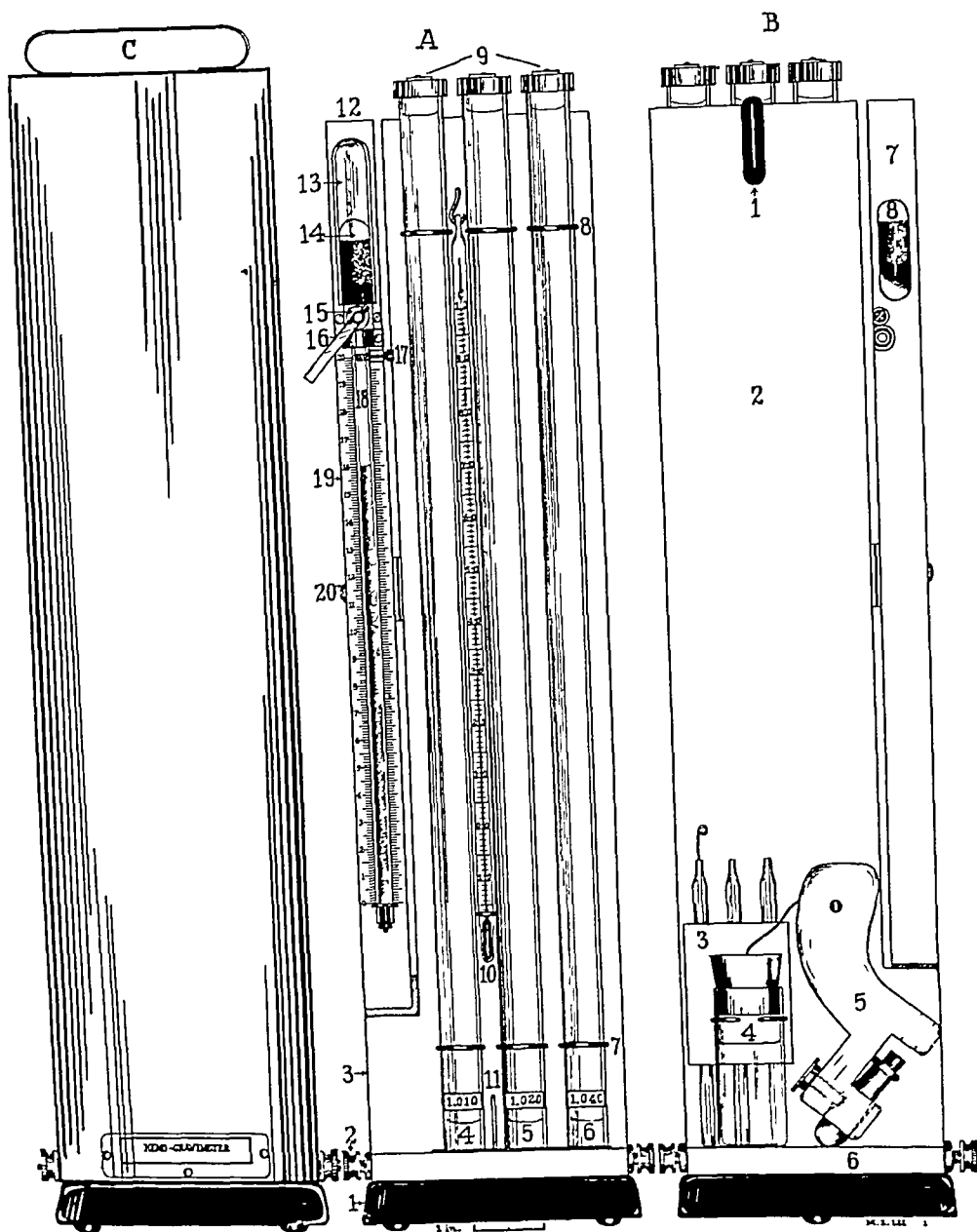


Fig. 1.—Hemo gravimeter or an apparatus for measuring specific gravity of watery fluids.
 A Front View 1 Iron base 2 cover locking nuts 3 enamelled wooden support 4 5 6 glass tubes containing gravity mixtures 7 8 supporting and timing rings 9 tube caps 10 thermometer 11 table and chart holder 12 timing device 13 sand reservoir 14 observation window 15 metal tip 16 start and stop lever 17 adjusting and stop screw 18 glass measuring tube 19 millimeter scale 20 fraction rotating support
 B Back View 1 Timg. handle for carrying 2 space for mounting tables and charts 3 pipette holder with pipettes 4 citrate rinsing solution 5 pipette controller 6 standard testing solutions (bottles not shown) 7 back of timer 8 observation window
 C Cover with carrying handle in place

TABLE I

SHOWING EQUIVALENT SPECIFIC GRAVITY FOR A GIVEN FALLING TIME, I E., A DPOP FALLING IN 11" AT 29° IN TUBE I, WOULD HAVE A SPECIFIC GRAVITY OF 1.029

TUBE I SP. GR.—1.0100

TIME SEC	SP. GR. AT TEMP. INDICATED								
	21°	22°	23°	24°	25°	26°	27°	28°	29°
10									
10.5									
11									
11.5							294	285	290
12					300	290	280	270	260
12.5				296	286	277	267	257	248
13			295	285	275	266	255	247	238
13.5		295	286	276	265	256	246	237	230
14	296	287	279	266	256	247	238	230	222
14.5	290	280	270	259	248	240	230	222	214
15	283	272	264	253	242	233	224	215	206
15.5	277	267	257	246	235	227	218	209	200
16	270	260	251	240	230	220	212	202	194
16.5	264	255	246	234	224	215	206	197	187
17	258	250	240	229	219	210	201	193	183
17.5	253	245	235	224	214	205	197	189	180
18	249	241	231	220	210	201	193	184	175
18.5	245	237	226	215	205	198	190	180	170
19	242	232	222	212	202	195	186	177	167
19.5	238	228	218	208	199	192	182	173	164
20	235	225	215	205	197	189	179	170	160

TUBE II SP. GR.—1.0200

TIME SEC	SP. GR. AT TEMP. INDICATED								
	21°	22°	23°	24°	25°	26°	27°	28°	29°
10	495	485	478	467	455	445	435	427	420
10.5	476	467	457	447	436	425	417	410	402
11	460	450	439	429	419	410	400	395	387
11.5	446	435	424	414	404	391	388	382	375
12	434	420	410	401	391	385	377	372	365
12.5	420	409	398	390	381	375	367	362	355
13	410	399	388	380	372	365	358	353	347
13.5	400	389	380	371	364	356	350	345	339
14	391	380	372	364	356	349	343	337	331
14.5	383	373	365	357	349	342	336	330	323
15	375	367	359	351	343	336	330	323	317
15.5	368	361	352	345	337	330	324	317	310
16	364	355	347	340	333	325	319	312	306
16.5	360	350	343	336	329	321	314	306	300
17	356	347	339	332	325	317	309	301	295
17.5	354	344	335	327	320	312	305	297	292
18	351	341	331	323	316	308	300	293	287
18.5	349	338	328	320	312	305	296	289	284
19	347	335	325	317	309	301	292	285	280
19.5	345	333	323	314	306	298	289	282	277
20	342	330	320	312	303	294	285	279	273

TABLE I (CONT'D)

TUBE III SP GR.—1 0400

TIME SEC	SI GR AT TEMP INDICATED								
	21°	22°	23°	24°	25°	26°	27°	28°	29°
10									395
10 5									575
11								590	575
11 5					600	588	575	560	545
12				595	587	577	564	548	533
12 5		600	592	585	577	565	552	538	525
13	600	592	584	576	567	556	542	530	515
13 5	592	584	576	568	559	547	535	522	507
14	585	577	567	561	551	539	526	513	500
14 5	577	570	561	553	543	532	518	505	493
15	572	564	555	547	537	523	512	498	487
15 5	566	558	549	542	530	516	505	492	481
16	561	552	543	535	524	510	498	486	475
16 5	556	547	537	529	518	505	492	481	470
17	551	542	534	523	513	500	487	476	466
17 5	546	537	528	519	508	495	483	472	461
18	542	533	523	513	503	491	478	467	457
18 5	537	529	519	509	499	486	475	463	453
19	533	525	515	505	495	482	470	460	450
19 5	530	520	511	501	491	478	467	455	
20	527	517	507	497	488	475	464	453	

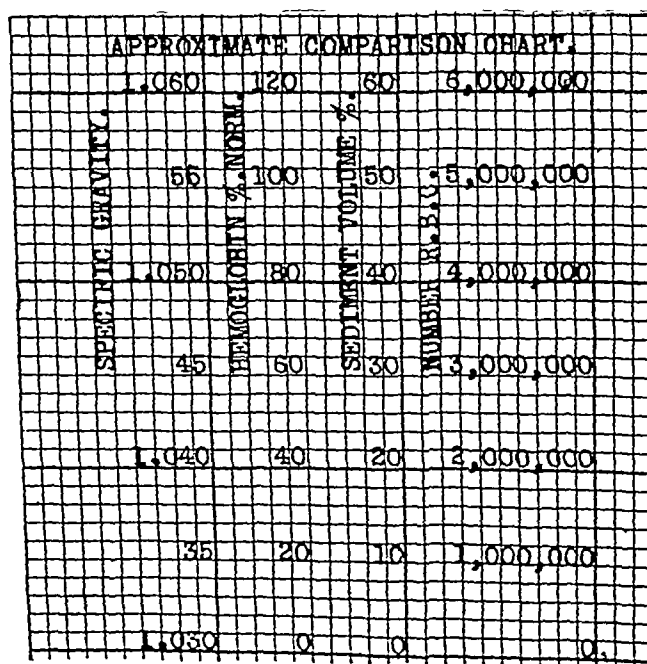


FIG. 2

By consulting the comparative chart, the gravity finding may be translated into terms of approximate hemoglobin content, sediment volume or red cell count (Fig 2)

Only a moment is required to rinse the pipette with citrate solution and to dismount it and return it and the controller to their places. The apparatus is provided with a cover and handle for carrying. Its dimensions are 4 by 4 by 18 inches and weighs five pounds.

Further details of technic and evaluation studies will be presented in a paper by McLain Lauler and Heintzelman.

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A RAPID PARAFFIN TECHNIC*†

W L McNAMARA, MD, HINES, IRL

WHILE the commonly employed methods for paraffin impregnation of tissues following rapid dehydration are suitable for surgical diagnoses, they cannot be used for minute histologic study or photomicrography. This is due to shrinkage of the tissue with resultant distortion and loss of cell definition.

The necessity for a rapid method yet one which would permit of detailed histologic study arose during a period of insufficient technical assistance and led to experimentation along these lines. This resulted in a method, which in our hands, has given highly satisfactory results and has been adopted routinely in this laboratory to the exclusion of all other methods.

The procedure is as follows:

10 per cent formalin fixation

Cut pieces for section 2 mm in thickness

80 per cent alcohol fifteen minutes

95 per cent alcohol forty-five minutes (40 x as much solution as tissue)

Acetone c p for fifteen minutes. Pour off and add 40 x as much fresh acetone as tissue for seventy-five minutes

Paraffin (hard 54°) in oven one hour. One paraffin only is used

Imbed and section

In removing paraffin from slide preceding staining, one xylol only is used

The usual staining processes may be employed

Sections are blotted following graded alcohols and cleared with some essential oil (cloves, organum, etc.)

*From the Clinical Laboratory and Laboratory Center and Cancer Center
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The secret of success in this method lies

1 In the avoidance of any of the commonly used clearing agents (xylol, chloroform, toluol, benzol, etc)

2 The least possible time in oven heat The clearing agents and prolonged heat are the factors largely responsible for tissue shrinkage

When no clearing agent other than an essential oil is used, paraffin impregnation takes place rapidly and is complete in less than one hour, thereby obviating long continued oven heat Acetone, being highly volatile, is rapidly driven off by the heat of the oven, hence, there is no necessity for more than one change of paraffin

The preceding technic carefully followed, results in rapid dehydration and paraffin impregnation with a minimum of tissue shrinkage and cell distortion

A PRACTICAL STAINING METHOD FOR INTESTINAL PROTOZOA*

II TSUCHIDA, Sc D, St LOUIS, Mo

AS CONTRASTED with the time consuming procedures employed for securing permanent preparations of intestinal protozoa by means of non-hematoxylin stain, the following method is found to be simple, rapid and efficient, especially when protozoa are required to be stained quickly for diagnostic work

The technic is described as follows

1 Smear a bit of stool on a clean slide, and make a thin moist film (thin enough to read newspaper print through) by using a drop of normal saline as diluent Keep the preparation moist at all times during this and subsequent steps This is vitally important

2 Fix immediately in warm Schaudinn's fluid (65 c c of saturated HgCl_2 and 35 c c of 95 per cent alcohol to which 3 c c of glacial acetic acid is added before use, and this is heated to 60°C) for ten minutes in case of trophozoites and fifteen minutes in case of cysts

3 Remove sublimate by immersing for ten minutes into 70 per cent alcohol to which several drops of the tincture of iodine are added

4 Wash in running tap water for one minute

5 Mordant in a 4 per cent aqueous iron alum (ferric ammonium sulphate) for twenty minutes

6 Wash in running tap water for three minutes

7 Shake off the excess water from the slide and immediately apply Wright's stain in a similar manner as for blood smear, i e, one minute with Wright's stain and five minutes after in addition of equal number of drops of distilled water

8 Wash in running tap water for one minute, and shake off the excess water from the slide

9 Dehydrate by graded alcohol fifteen seconds each in 70 per cent, 95 per cent, and absolute alcohol

10 Clear in two changes of xylol for three minutes each

11 Apply a coverslip gently over a drop of neutral balsam, and leave in an incubator at 37° C until examined

Contrary to the hematoxylin methods, this method does not require differentiation of Wright's stain by means of a mordant, the process of which is considered difficult by those who are inexperienced. Furthermore, Wright's stain gives a very clear picture, and enables one to recognize readily the structural characteristics of an organism in question. Such may be well illustrated by the nuclei of various endamebae in which the karyosome, chromatin granules, and linen network are brought out in dark blue color against a pinkish background of eosin.

By virtue of the simplicity and the brevity of time spent for the technic together with vivid color contrasts of various structures, this method can advantageously be employed in a final diagnosis of intestinal protozoa, whenever it is difficult to determine with certainty the species present.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

LEUKEMIA Proteolytic Leukocytic Enzyme in, Cooke, J. V. Arch. Int. Med. 49: 836, 1932

The following method was used:

At approximately the same time that a total and stained differential leucocyte count is made, a sample of venous blood is placed in a test tube with a small amount of powdered sodium oxalate to prevent clotting. The total volume having been marked on the tube, the specimen is then centrifuged and the supernatant plasma removed and discarded, care being used to avoid taking away any cells. The cells are then washed three times in 0.85 per cent salt solution to get rid of the last traces of serum, and after the last washing the same solution is added to the tube until the original volume is reached. This is well shaken to distribute the leucocytes evenly, and 3 c.c. samples are placed in large test tubes with a volumetric pipette. These are treated as follows: (1) Control. To this tube is added 21 c.c. of distilled water, 3 c.c. of a 10 per cent solution of sodium tungstate and 3 c.c. of two thirds normal sulphuric acid. After shaking and filtering, the nonprotein nitrogen is determined on a sample of the filtrate and the milligrams of nonprotein nitrogen per hundred cubic centimeters calculated. (2) Protease activity in neutral medium. After the addition of 7 c.c. of water and a small quantity of toluene, the tube is incubated at 37° C. for five days. Then 3 c.c. of a 10 per cent solution of sodium tungstate, 3 c.c. of two thirds normal sulphuric acid and 14 c.c. of water are added and the nonprotein nitrogen determination made on the filtrate.

The procedure outlined is the well known method of Folin and Wu and the determinations of nonprotein nitrogen are carried out by acid digestion and nesslerization, as usual. The results are all expressed as milligrams of nitrogen per hundred cubic centimeters of blood.

Samples of blood without leucocytes obtained by taking only the lowermost layers of cells after washing with salt solution by centrifugation show no evidence of proteolytic activity.

In chronic myeloid leucemia the blood protease was found to be considerably increased. Although the readings usually varied in direct relation to the leucocyte count, there were certain exceptions suggesting that the protease activity of these cells might vary under certain conditions.

In the single case of chronic lymphoid leucemia studied the blood protease was decreased.

In acute lymphatic leucemia, both high and low values for blood protease were found. An analysis of twenty determinations made on twelve patients indicates that the non granular leucocytes in acute leucemia may have a rather high protease activity comparable to that of the granular cells but that this protease content becomes markedly decreased in the late stage of the disease. A similar decrease in protease activity of these cells accompanies toxic periods shown by fever and prostration which may occur in the course of the illness.

The fact that the abnormal nongranular leucocytes of acute leucemia may contain abundant protease even though their enzyme content may be decreased in certain stages of the disease is interpreted as evidence that these cells should be considered more closely related to the granular myelocytes than to the true lymphocytes, and that their origin is in the bone marrow and not in the lymphatic system. It seems probable that the essential site of disturbance in acute ("lymphatic") leucemia is the bone marrow and not the lymphatic system.

MENINGITIS Tuberculous, Levinson Test in, Gleech, M. *Am J Dis Child* 43 1077, 1932

The author considers the test of corroborative value

BLOOD SEDIMENTATION Practical Value of, Cutler, J W. *Am J Med Sci* 183 612, 1932

Based on a study of 5000 patients the following conclusions are drawn

DISEASES GROUPED ACCORDING TO SEDIMENTATION RATES

With an Abnormal Sedimentation Rate

- 1 Chronic infectious disease, such as tuberculosis and syphilis
- 2 Acute infectious diseases, such as pneumonia, septicemia, acute endocarditis, the exanthemata and acute bronchitis
- 3 Malignancy
- 4 Localized suppurations, such as pelvic inflammatory disease, suppurative mastoiditis, suppurative sinusitis, empyema of the gall bladder, bronchiectasis
- 5 Acute intoxications, such as lead and arsenic poisoning
- 6 Certain endocrine disturbances, such as thyroid toxicosis

INFLUENCING THE SEDIMENTATION RATE VERY LITTLE IF AT ALL

- 1 Simple catarrhal inflammations, such as acute catarrhal appendicitis, simple rhinitis and colitis
- 2 Chronic ulcerations of small extent such as gastric or duodenal ulcer

NOT INFLUENCING THE SEDIMENTATION RATE

- 1 Functional diseases, such as the various neuroses and neurasthenia
- 2 Certain nervous diseases, such as dementia praecox
- 3 Focal infections, such as abscessed teeth, diseased tonsils and chronic sinusitis
- 4 Metabolic diseases, such as uncomplicated diabetes and essential hypertension
- 5 Allergic diseases, such as asthma and hay fever
- 6 Most skin diseases
- 7 Simple growths, such as fibroma, lipoma, and fibromyoma
- 8 Simple cysts
- 9 Chronic valvular disease of the heart

ECLAMPSIA Serum Calcium in, Anderson, D F. *Brit J Exper Path* 13 182 1932

Eighty-two per cent of the 44 cases of eclampsia investigated showed subnormal serum calcium values, that is, 9 mg per 100 cc and under

Fifty-nine per cent of the 17 cases of nephritic and preeclamptic toxemia were associated with a subnormal serum calcium

Seventeen per cent of the 35 cases of normal pregnancy at full time, in labor, and shortly after delivery had serum calcium values lower than normal

Thirty-one other cases, mainly of complicated pregnancy, were examined in respect of their serum calcium level

From the available data, it is impossible to assess the effect of injections of calcium gluconate, intravenously or otherwise. In the doses given, 10 cc it has no abrupt effect on the frequency of the fits

BILIRUBINEMIA Qualitative and Quantitative Estimation of, Greco, A. *Diag & Tech Lab* 2 925, 1931

Greco describes a modification of Doddi's method as follows

Reagents

- 10 per cent solution barium chloride
- N/10 alcoholic solution potassium hydroxide
- Concentrated sulphuric acid

Doddi's reagent

- Potassium isoparamitrodiazobenzol 5 parts
- Distilled water 1000 parts
- Must be freshly prepared

ABSTRACTS

METHOD

To 7 cc of *fresh acid* urine add 3 cc of barium chloride solution. Mix, centrifuge, discard supernatant fluid and wash the precipitate several times with distilled water. Add 2 cc of potassium hydroxide solution and shake for one minute. Centrifuge and decant supernatant fluid to a clean tube.

Add 6 drops of freshly prepared Dodd's reagent and 0.5 cc of sulphuric acid. Bilirubin gives a sudden reddish purple color turning purple and become more intense in a few seconds.

The test is sensitive to 1:1,000,000.

The reaction may be made quantitative by using a special diagram adjusted to the Autenreith colorimeter.

PRECIPITATION TEST A New Kahn Antigen Mixer, Gurchot, C. Science, 75: 340, 1932

The materials used are as follows:

1. A specimen vial $\frac{1}{4}$ inch inside diameter by $2\frac{1}{4}$ inches long.

2. One rubber stopper No. 3 with 2 holes.

3. A 1 inch funnel with a stem 4 inches long.

4. A $1\frac{1}{2}$ inch right angle glass tube, $\frac{1}{8}$ inch inside diameter.

5. A piece of rubber tubing 9 inches long by $\frac{3}{8}$ inch diameter with glass tubing mouth piece.

The end of the funnel is heated until the hole is $\frac{1}{2}$ mm in diameter. When inserted through the rubber stopper and the vial closed the funnel stem should almost touch the bottom. For greater stability the base of the vial can be held in a No. 9 rubber stopper.

The antigen is put into the vial first. After the stopper is replaced, gentle suction by mouth or otherwise is applied by means of the rubber tube connected with the right angle glass tube. This causes bubbling through the antigen. Now the salt solution is poured into the funnel one drop at a time, say 2 or 3 drops per second. The entrapped air between the drops does the mixing of the salt solution with the antigen in the vial. If mechanical suction is used and also a dropping funnel, it is possible to reproduce the mixing exactly each time and standardize the emulsion with great accuracy, something almost impossible with the mixers in general use.

MENINGOCOCCUS STRAINS Observations on the Serological and Immunological Reactions of, in Relation to Serum Production, Kirkbride, M. B., and Cohen, S. M. (Author's abstract)

In a study of meningococcus strains made in connection with the production of therapeutic antimeningococcus serum, the results of the classification of 441 strains received from 1916 through 1930 were summarized as follows: Of 115 strains received from 1916 through 1927, 87 per cent were Group I, 33 per cent, Group II, 24.3 per cent, Group III, 0.9 per cent, Group I or III, and 33 per cent, Group "X." Of 326 strains received from 1928 through 1930, 24.5 per cent were Group I, 3.9 per cent were Group II, 15.3 per cent, Group III, 28.2 per cent, Group I or III, and 27.9 per cent, Group "X."

No strains belonging to Group IV were found in this series. However, since strains of this group had been isolated in a recent epidemic in this country, a comparison of several Group IV strains was made. The American Group IV, as represented by the federal standard strain, did not appear to be related to the English Group IV according to the results of agglutination tests. A strain isolated in this country in 1928 was found to be allied with both the federal and English strains and may represent a broad group which includes all three. On account of their limited incidence, it is questionable whether Group IV strains are essential at present in the production of therapeutic sera.

A polyvalent serum produced with six carefully selected strains, representative of Groups I, II and III appeared to possess values for the majority of the strains classified as Group "X," which included strains not specifically agglutinated by the monovalent group sera.

The strains of Groups I and III which gave group specific serologic reactions when selected as standard strains in 1919 have become, as a result of long continued artificial cultivation very closely related in agglutination and agglutino-genic reactions. However, since cer-

from freshly isolated strains are agglutinated specifically by either Group I or Group III sera, it is considered important for purposes of therapeutic serum production to continue to recognize both groups. Federal standard strains of Groups I and III were also found to be closely related. They did not, however, correspond closely in serologic reactions to state standard strains of the same groups.

None of seventeen recently isolated meningococcus strains received from epidemic centers, was apparently markedly superior antigenically, as determined by the agglutinative titers of sera produced in rabbits, to the state standard strains used for more than thirteen years in serum production.

Further laboratory study and clinical data are required before the present state strains are replaced by recently isolated strains in the routine production of the therapeutic serum.

URINE A New Method of Staining Urinary Sediment, Fiorentino, M. Diag. e Tech. di Lab. 2 847, 1931

Fiorentino describes a method devised by himself as follows:

Reagents—

I The following stock solutions are needed, all being stable:

- a Saturated aqueous solution of methyl violet
- b Saturated aqueous solution of toluidine blue
- c Saturated aqueous solution of Nile blue (sulphate)
- d Saturated aqueous solution of cresyl-violet

II Sudan III (powder)

III A 10 per cent solution of sodium chloride to which is added 30 per cent of acetone

NaCl	200 gm
Distilled water	1400 cc
Acetone	600 cc

The formula for the staining solution is as follows:

Stock solution a	0.4 cc
Stock solution b	3.0 cc
Stock solution c	3.6 cc
Stock solution d	60.0 cc
Acetone chloride solution	133 cc

Place in a well stoppered bottle, add Sudan III to saturate, shaking at frequent intervals for "several" days.

Allow the undissolved Sudan III to settle, decant the supernatant fluid and filter rapidly through a double filter well moistened with the acetone chloride solution. Add to the filtrate 20 cc of the acetone chloride solution.

Method—

To a minute drop of sediment on a clean slide add a large drop of the stain and mix rapidly with a glass rod, smearing the mixture at the same time with a rotary motion.

Apply cover glass and examine.

A colored plate shows the staining reactions.

COLLOIDAL GOLD SOLUTION Method for Preparation of, Levine, B. S. Am. J. Syph. 16 103, 1932

The glassware required: Two 1000 cc Florence flasks so built that 1000 cc of fluid does not quite fill their bulbs; two 10 cc, one 5 cc, and one 1 cc pipettes; one 1000 cc and one 500 cc cylinder graduates.

The reagents: Gold chloride, 1 per cent solution; potassium carbonate, 2 per cent solution; formaldehyde, fresh, 1 per cent solution; hydrogen peroxide solution, absolutely fresh. All chemicals must be of the highest degree of purity, and the solutions must be prepared with the utmost degree of accuracy.

Cleaning of glassware: Fill the Florence flasks and the cylinders with aqua regia, submerge the pipettes into this cleaning mixture in one of the cylinders and let stand overnight.

The following morning pour the aqua regia into the storage bottle and rinse the glassware inside and out twice with carefully prepared singly distilled water, taking care to remove all the acid vapor. Now, fill the flasks and the cylinders with a 1 per cent solution of Na_2CO_3 made with singly distilled water and submerge the pipettes into one of the cylinders. Rinse the glassware with this alkaline solution inside and out thoroughly. Pour the carbonate solution into the storage bottle and rinse the glassware twice with singly distilled water. The same procedure of cleaning should be applied to the bottles in which the reagents are made up, to the tubes in the test proper, and to the glassware used for making the distilled water. This step of glassware cleaning is essential and should be followed out scrupulously.

Preparation of the gold sol. Measure into each of two Florence flasks, cleaned as described above, 500 cc of carefully prepared doubly distilled water. Heat one flask to 90° and the other to 60° . Remove from the flame. To the flask heated to 90° add 10 cc of the 1 per cent gold chloride solution. Heat for about one minute. Remove from the flame. Add 7 cc of the 2 per cent potassium carbonate solution. Shake until the golden yellow color completely disappears.

To the Florence flask containing the 500 cc of doubly distilled water heated to 60° add 5 cc of the 1 per cent formol solution. Shake well. Now, with the 1 cc pipette add to this flask from one to three drops of absolutely fresh hydrogen peroxide. Shake the contents to insure thorough distribution of the reagents. Rapidly and with constant shaking add the formol peroxide solution to the gold carbonate solution. Continue shaking until the reaction is complete.

The progress of the reaction is rapid and is denoted by the successive appearance of three colors. First, a light violet, second, a blue to dark blue, third, a rose red to ruby red with a tinge of blue. Occasionally the completion of the reaction is more direct and the blue color does not appear. Aerosol thus prepared appears transparent by transmitted light and decidedly laky by reflected light. Five mls of it become completely decolorized by 17 cc of a 1 per cent NaCl solution within fifteen to sixty minutes. With cerebrospinal fluids such aerosol gives regular results of the proper sensitiveness and reliable constancy.

STAIN Method for Spirochetes and Moulds With Anilin Dyes, Olsen, R. E., and Weller, C. V. *Am J Syph* 16: 113, 1932

A. Phosphomolybdic Acid Dye Method for Smears—

- 1 Dry unfixed smear preparation by flaming gently
- 2 Immerse in a saturated aqueous solution of phosphomolybdic acid at 50° to 65° C for thirty to sixty minutes
- 3 Wash off in distilled water for a few seconds
- 4 Immerse in the staining solution at 50° to 65° C for thirty to sixty minutes. Carbol fuchsin, carbol iodine green, and Unna's alkaline methylene blue are recommended but many other basic aniline dyes may be used
- 5 Wash off in distilled water, dehydrate in absolute alcohol, transfer to xylol, and mount in Canadai balsam, or after washing simply dry by blotting and mount in balsam

B. Phosphomolybdic Acid Dye Method for Cover Slip Sections of Fixed Tissues—

- 1 Cut paraffin sections about ten microns thick and mount on clean cover glasses with albumin fixative
- 2 Dry in oven at about 40° C for two to twenty four hours
- 3 Remove paraffin in xylol and transfer through absolute alcohol to distilled water
- 4 Proceed with the method given for smear preparations

TUBERCULOSIS Parenteral BCG Vaccination, Kerezturni, C., Park, W. H., and Shick, B. *Am J Dis Child* 43: 27, 1942

The following conclusions are advanced from this study:

Intradermal BCG vaccination is superior to the subcutaneous type, because if the technique is correct no cold abscess develops. On the other hand, hypersensitivity to tuberculin occurs a little more frequently and lasts somewhat longer when the subcutaneous method is employed.

In 87 per cent of parenterally vaccinated patients hypersensitiveness to tuberculin developed either temporarily or for a longer period

The use of both the subcutaneous and the intradermal methods of BCG vaccination has been harmless

CANCER The Bendien Test for, Freeman, M., et al. Med J Australia 11 778 1931

From a study of this test in 277 cases the authors conclude that the reaction is non specific and unreliable

BRUCELLA GROUP Serological Differentiation of Smooth Strains, Wilson, G S, and Miles, A. A. Brit J Exper Path 13 1, 1932

As a result of the work recorded in the present paper, and in a paper by Pandit and Wilson (1932), it is concluded that the Brucella group contains members which may be primarily classified into smooth and rough

The smooth strains, comprising abortus of bovine and porcine origin and melitensis, are non thermo agglutinable, and though sometimes agglutinated slightly by acid, are not agglutinated by silt. The rough strains, comprising parabortus and paramelitensis are thermo agglutinable, are agglutinated strongly by acid, and not infrequently by silt

In their typical forms smooth and rough strains have no serologic relationship, though intermediate strains occur containing both smooth and rough antigen

By the use of sera prepared against absolutely smooth strains, it is possible by the agglutinin absorption technique to divide the smooth members into two types one type containing bovine and porcine abortus, the other type containing melitensis strains

Evidence is brought to suggest that the distinction between abortus and melitensis strains is due, not to the presence of qualitatively different antigens, but to the different quantitative distribution of two common antigens

Provided due regard is paid to the relationship between the absorbing dose and the titer of the serum, monospecific sera can be prepared in which the major agglutinin of the type alone persists. By means of these sera unknown strains of the Brucella group can be rapidly typed by direct agglutination

The results of testing one hundred strains by monospecific sera are recorded, and with a single exception afforded by a group of strains from a particular locality, are seen to be in close accord with conclusions reached on epidemiologic and other grounds

The rough strains have not been fully studied, but it appears that there is at least one antigen common to all parabortus and paramelitensis strains

It is suggested that the reason why so many previous workers have failed to differentiate serologically between abortus and melitensis is because they have not realized the importance of using absolutely smooth strains for the preparation of their sera. Since melitensis strains have a marked tendency to become rough in the laboratory, it is, as a rule, difficult to obtain satisfactory sera against the melitensis type unless recently isolated strains are used. Unless perfectly smooth strains are employed, the resulting sera will contain some rough agglutinin, which will tend to obscure the clear differentiation of the types

BILIRUBINEMIA The Diazo Reaction as a Quantitative Procedure, White, F D. Brit J Exper Path 13 86, 1932

The following new standard is described

One and three tenths grams of anhydrous cobalt sulphate are dissolved in 50 cc of distilled water. To this is added gradually, with shaking and cooling, 40 cc of concentrated hydrochloric acid (sp gr 1.19), and the solution made up to 100 cc with distilled water. As the acid is added the color of the solution changes to a bluish violet, which gradually reverts to a more reddish violet, the permanent hue. For this reason the solution should be prepared twenty four hours before use, and kept well stoppered and out of contact with light. Prepared thus, the solution is apparently stable, it has been tested repeatedly during a period of three months, and has invariably shown the same azobilirubin color value. Further, the color intensity of the solution is proportional to the concentration of cobalt salt, and consequently weaker or stronger standards can be prepared by dissolving the appropriate amounts of the

sulphite, adding 40 cc of concentrated hydrochloric acid, and making up to 100 cc, although the color is such that stronger standards are not recommended

MENINGITIS New Reaction in Spinal Fluid, Friedman, A P Arch f Psychiat 95 273 1931

To 1 cc of freshly withdrawn cerebrospinal fluid, 1 drop (0.05 cc) of a 1 per cent aqueous solution of potassium permanganate is added, and this mixture is well shaken. In normal cerebrospinal fluid or in patients with organic disorders of the central nervous system without involvement of the meninges, the mixture has a light violet color and this color persists, even if from 2 to 3 drops of a 20 per cent solution of trichloroacetic acid is added. However, in cases of meningitis the violet color changes a few seconds after addition of the potassium permanganate solution to a rose yellow and to a brown yellow, and if trichloroacetic acid solution is then added to the cerebrospinal fluid of purulent meningitis the reaction goes still further: the potassium permanganate becomes more decolorized. The mixture becomes light yellow and finally entirely colorless with simultaneous clouding and sediment formation. In other forms of meningitis the latter changes are not noted.

B. MUCOSUS INFECTION of the New Born, Jampolis, M., et al Am J Dis Child 43 70, 1932

An outbreak of infectious diarrhea developed in a nursery for the newborn. This outbreak spread insidiously. A latent period of two months elapsed after the first few cases appeared.

The constitutional symptoms, severe intoxication, dehydration and prostration, were out of proportion to the relatively mild diarrheal symptoms.

The stools averaged about six daily. They were watery and contained mucus but no blood or pus, except in one case.

The mortality was high in spite of the usual accepted treatment for anhydremic intoxication.

Apparently the offending organism was *B. mucosus*, the virulence of which may have been enhanced by symbiosis with anhemolytic streptococci. *B. mucosus* was isolated from the nasal secretions, stomach contents, stools and intestinal mucosa in a large number of the cases. None of the usual organisms causing infectious diarrhea, such as the typhoid dysentery groups, were found.

The primary and outstanding pathologic findings in the fatal cases consisted of acute enteritis, the mucous membrane of the ileum being red, swollen, finely granular and covered with reddish gray mucus. Microscopic examination showed the mucosa to be infiltrated with polymorphonuclear leucocytes and lymphocytes. A few shallow ulcers were found and the lymphoid tissue was hypertrophied. However, there was a relative absence of involvement of the colon, which probably accounts for the comparatively few diarrheal stools and the absence of pus and blood.

In only a few instances was there evidence of parenteral infection. In a few babies terminal bronchopneumonia and otitis media developed. Cultures from discharge of the ears and the lungs in these cases revealed the same organisms as were found in the intestinal lesions, namely, *B. mucosus* and anhemolytic streptococci.

Repeated cultures from the throat and stool of three nursery maids revealed practically pure cultures of *B. mucosus*. When these nursery maids were relieved of their duties in the nursery, the outbreak promptly subsided, and there has been no recurrence during the past nine months. Cultures from two of these girls became negative a few weeks after tonsillectomy. The third one refused operation, and the cultures remained positive.

For the past few years pediatric literature has contained numerous accounts of outbreaks of diarrhea similar to the one described here. They are usually attributed to parenteral infections. For the most part, respiratory diseases and otitis media. The parenteral infection is usually held responsible for the severe general symptoms, and the diarrhea is considered secondary and incidental. The investigations reported in this article apparently point out that diarrhea accompanied by marked prostration, dehydration and intoxication may be due to primary enteritis even though the stools do not show pus or blood.

The outstanding features reported are (a) *B. mucosus*, as the etiologic agent in severe infectious diarrhea, (b) nursery mounds as carriers, (c) the relative absence of preterminal infections, and (d) the absence of the bloody purulent stools that are considered characteristic of infectious diarrhea.

AGRANULOCYTOSIS Myeloid Cell Hyperplasia in Bone Marrow, Fitzhugh, T, and Krumbhaar, E B. *Am J M Sc* 183 104, 1932

Myelocytes and myeloblasts were found in the bone marrow at necropsy in more than normal numbers in a case of typical "granulocytic angina" whose antemortem blood count was 200 white cells per c mm (all lymphocytes), i. e., a marked absolute reduction of lymphocytes and absence of all other white cells.

Based on this and similar cases recorded in the literature, objection is raised to the current hypothesis of "granulocytic aplasia" as constituting the "primary" pathologic mechanism of the disease, and in its place in hypothesis of "maturation arrest" is proposed for consideration and future study.

Inasmuch as there is an absolute reduction of lymphocytes in the blood stream as well as of neutrophils and on account of certain analogies with pernicious anemia, designation such as pernicious leucopenia is suggested as preferable to the more widely used names for this disease.

MALARIA Determination of Quinine in the Blood as a Guide to Treatment, Vedder, E B, and Masen, J M. *Am J Trop Med* 11 217, 1951

Two methods are described as follows:

METHOD I PREPARATION OF REAGENTS AND STANDARDS

1. A 10 per cent solution of silicotungstic acid in distilled water.
 2. A 0.5 per cent normal solution of hydrochloric acid.
 3. Quinine standards. A stock solution is prepared containing 200 mgm anhydrous quinine in 1000 cc of 0.5 N HCl. One cubic centimeter of this solution contains 0.2 mg.
- From this four standard solutions are prepared all dilutions being made with 0.5 N HCl.
1. Five tenths cubic centimeter stock solution is diluted to 100 cc. Five cubic centimeters of this solution contain 0.005 or 1 mg per liter.
 2. One cubic centimeter stock solution diluted to 100 cc. Five cubic centimeters contain 0.01 mg, 2 mg per liter.
 3. Two cubic centimeters stock solution to 100 cc. Five cubic centimeters contain 0.02 or 4 mg per liter.
 4. Three cubic centimeters stock solution to 100 cc. Five cubic centimeters contain 0.03 or 6 mg per liter.

These standards cover routine procedure enabling estimations of quinine in the blood from 1 to 8 mg per liter. Higher standards may be prepared as required. These solutions deteriorate when exposed to light and must be kept in brown glass bottles. If protected from the light they are permanent for at least one year.

Technic—A small circle of absorbent filter paper cut from a thick Whittman extraction thimble is placed in the bottom of tube "a" of the extraction apparatus which may be made in any laboratory. This tube is then packed with long fiber, acid washed asbestos to within about 2 cm of the top. The asbestos must not be packed too tightly, otherwise the ether will not percolate through the blood properly. Neither must the packing be too loose or the blood will not be properly absorbed on the asbestos and some will escape to the bottom of the tube into the ether. The constriction is placed in tube "a" to prevent blood from flowing down the walls of the tube thus escaping absorption by the asbestos.

Five cubic centimeters of oxalated blood is pipetted on to the asbestos of a small pledget of cotton placed in the mouth of the tube, which is then inserted in tube "b". Ether is poured through the top of tube "a" until at least 5 cc has percolated into the outer tube "b". The extractor is connected to a reflux condenser and is immersed in a warm water bath up to the level of the ether in tube "b". The extraction is then allowed to proceed for two hours.

When extraction is complete, the inner tube is removed, and the outer tube containing the ether extract is placed in a boiling brine bath and evaporated to dryness. Five cubic centimeters of 0.5 N HCl is added and the tube returned to the brine bath for two minutes to facilitate solution of the gumme. The solution is filtered while hot, through a No. 42 Whatman filter, and the filtrate allowed to cool to room temperature.

Three standards are prepared equivalent to 2, 4, and 6 mg. per liter by measuring 5 cc. of the proper standard solutions into small test tubes. To each of the standards is added 0.1 cc. of the 10 per cent solution of silicotungstic acid, and to 3 cc. of the unknown is added 0.06 cc. of the same solution. Standards and unknown are then immersed together in a boiling water bath for five minutes, after which they are removed and cooled rapidly in running water. The unknown is then matched in the nephelometer against the nearest approximate standard.

METHOD 2 PREPARATIONS OF SOLUTIONS AND REAGENTS

1 *Gum Arabic*—Two grams of pure gum arabic (U. S. P. is suitable) is shaken with 100 cc. of distilled water until completely dispersed. The suspension in a Florence flask is immersed in a boiling brine bath for one hour to destroy reducing enzymes present in the gum, and is then filtered while still hot. On cooling the gum is ready for use, and will keep indefinitely if stoppered and kept in a refrigerator. It keeps about two months in the laboratory.

2 *A Stock Standard* is prepared by dissolving 100 mg. of pure anhydrous quinine in 500 cc. of 2 N sulphuric acid saturated with zinc sulphate. The working standards are prepared by diluting the stock solution 1:100, 2:100, 3:100 etc., with 2 N sulphuric acid saturated zinc sulphate solution. Five cubic centimeters of these dilutions contain 0.01, 0.02, and 0.03 mg. quinine respectively or 2, 4, and 6 mg. per liter. These standards must be protected from the light by brown glass bottles. As the sensitivity of the test is affected by the concentration of the zinc sulphate, the zinc sulphate sulphuric acid solution for the preparation of the standards should be the same as that used for dissolving the quinine extracted from the blood.

3 *Two N Sulphuric Acid Zinc Sulphate Solution*—The purest crystalline salt, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, should be used. The 2 N sulphuric acid should be saturated with the salt at temperatures somewhat below those encountered in the ordinary room temperature, as otherwise trouble will be experienced on account of the zinc sulphate crystallizing out as soon as the room temperature drops. Thus, if the minimum laboratory temperature is 20°C the solution should be saturated at a temperature of 15° to 17°C and should then be filtered.

4 *Potassium Bismuthous Iodide Reagent*—This is a reagent commonly used in the detection of alkaloids, and there are a number of formulas for its preparation. The one outlined gave the best results. Place 4.68 gm. of bismuth oxide in 80 cc. concentrated HCl, and add water to 300 cc. Then dissolve 20 gm. potassium iodide in water and dilute to 700 cc.

Technic Extraction of the blood is performed as described in Method I. As soon as extraction is complete and the ether is evaporated, 5 cc. of 2 N sulphuric acid saturated with zinc sulphate is added, and the tube immersed in the boiling brine bath for three minutes in order to bring the quinine into solution. The solution while hot, is filtered through a No. 42 Whatman filter, and, after having cooled to room temperature, an aliquot of the filtrate, 3 cc., is measured into a small test tube. In three similar test tubes measure 5 cc. of each of the three quinine standard solutions. Immerse both standards and unknown in a cold water bath (20 to 25°C) for five minutes in order to bring them to the same temperature. Then add to the standard 0.1 cc. and to the unknown 0.06 cc. of the gum arabic solution, followed by the same amounts of the potassium bismuthous iodide reagent (0.1 cc. to the standard and 0.06 cc. to the unknown). Mix and compare immediately in the colorimeter, with the standard set at 10 mm. The comparisons must be made within two minutes from the time that the solutions are mixed, as the color changes on standing. The calculation is made in the usual manner as described under Method I.

As in most colorimetric procedures, the closer the strength of the standard approximates that of the unknown, the more accurate will be the colorimetric matching. However for practical purposes it is sufficient to prepare three standards as just described and comparing the unknown with the nearest standard.

RETICULOCYTES Staining of, Osgood, E. E., and Wilhelm, M. M. *Proc Soc Exper Biol & Med* 29 5, 1931

The following method was found satisfactory with oxidized blood

Mix equal parts (5 drops) of oxidized venous blood (or fresh blood) and 1 per cent brilliant cresyl blue in 0.85 per cent NaCl solution in a small test tube and allow to stand one minute or more. Mix and make a thin smear. This may be counted when dry, or counterstained with Wright's stain. Count all the red cells, (preferably with a hand tally) in an oil immersion field and then count all the reticulocytes in that field. Move to an adjacent field and repeat until 1000 red cells have been counted. If the count is more than 5 per cent, only 500 cells need be counted. The counterstain is necessary if the slide is to be kept for more than forty-eight hours.

This method has many advantages. It is very simple and convenient and consistently gives a higher reticulocyte count than the other methods tried. The stain keeps indefinitely and need not be filtered before using. The oxidized blood may stand for as long as forty-eight hours before the count is made. Overstaining does not occur even though the smears are not made until two hours after the stain and blood are mixed. The reticulocytes are clearly and deeply stained and the red cells are neither crinkled nor distorted. The smears keep indefinitely if counterstained with Wright's stain.

Preliminary studies suggest that bloods of healthy adults will show about 2 per cent reticulocytes.

GOITER The Blood Picture in, Jackson, A. S. *J A M A* 97 1954, 1951

In a study of 600 cases of goiter, the following conclusions are drawn:

1 The blood picture in hyperthyroidism does not vary essentially from that in the normal person.

2 The differential blood count in hyperthyroidism is not of diagnostic and prognostic significance.

3 There is not a definite relationship between the blood picture and the basal metabolic rate. The lymphocyte count is not varied by an increase or a decrease in metabolism.

4 The blood count is not influenced by the severity of the disease, considering metabolism and weight loss as paramount factors.

5 A secondary anemia is not typical of hyperthyroidism.

6 A leucopenia is not characteristic of hyperthyroidism.

7 Sex and age do not influence the blood picture in toxic goiter.

8 In spite of clinical improvement, no appreciable change was observed in the blood count following the use of iodine in hyperthyroidism.

9 The only appreciable change in the differential blood count in goiter was observed following thyroidectomy for exophthalmic goiter. An increase in the polymorphonuclear count and a decrease in the lymphocyte count occurred.

The authors do not believe that the blood picture in hyperthyroidism is of any practical clinical importance.

ARTHRITIS Bacteriologic Investigations in, Dawson, M. H., Olmstead, M., and Boots, R. H. *Arch Int Med* 49 17, 1932

The authors reported the work of Cecil, Nicholls, and Stansby in a study of 80 patients and present the following conclusions:

1 One hundred and five blood cultures, the majority in duplicate, were carried out on 80 patients suffering from rheumatoid arthritis according to the technique of Cecil, Nicholls, and Stansby. As control material, 31 samples of blood from normal persons and 16 samples of sterile inoculated agar were subjected to similar manipulations.

2 The blood cultures on patients suffering from rheumatoid arthritis failed to yield organisms that could be considered of etiologic significance.

3 No significant difference was observed in the bacteria encountered in the blood cultures of patients and those observed during the culture of the control material under similar conditions.

4 *Streptococcus viridans* was occasionally encountered during the culture of the control material as well as during the culture of specimens of the patients' blood

5 Aerobic and anaerobic cultures of 23 specimens of synovial fluid obtained from patients suffering from rheumatoid arthritis failed to yield organisms that could be considered of etiologic significance

6 Aerobic and anaerobic cultures of 12 subcutaneous nodules obtained from patients suffering from rheumatoid arthritis failed to yield organisms that could be considered of etiologic significance

RETICULOCYTES The Response of, to Iron, Minot, G R., and Heath, C W Am J M Sc 183 110, 1932

A study is presented concerning positive reticulocyte responses to the daily oral administration of iron in maximal amounts to patients with anemia especially due to chronic blood loss, dietary defects, gastrointestinal disorders and pregnancy and to patients with chronic microcytic anemia of obscure origin

The height of the reticulocyte rise is in general inversely proportional to the level of the red blood cells and hemoglobin directly before treatment, but the relationships are less exact for the anemia responding to iron than for pernicious anemia in response to liver or potent substitute

Infections and other complications hinder the action of iron similar to the way in which they hinder the effect of potent material for pernicious anemia

Distinct rises of reticulocytes occur with low hemoglobin values in response to iron when the red blood cell level is one at which in pernicious anemia insignificant reticulocyte increases take place

With red blood cells above 2.5 million per cmm a greater rise of reticulocytes occurs in response to maximal amounts of iron than in pernicious anemia in response to adequate amounts of potent material, but when the red blood cells are below this number the reticulocytes rise in response to adequate therapy to a somewhat similar number in the different types of anemia On the contrary, in pernicious anemia as the hemoglobin level decreases below about 10 gm per 100 cc of blood the rise of the reticulocytes becomes progressively greater than in "secondary" anemia, so that it is at least double when the hemoglobin is less than about 5 gm per 100 cc of blood

Both the hemoglobin and red blood cell levels must be considered in evaluating the reticulocyte response to iron For a given red blood cell level the reticulocytes will increase more the lower the hemoglobin and the increase of reticulocytes will be greater at a given hemoglobin level the lower the red blood cell count

The exact type of case responding to iron plays a rôle in the degree of reticulocyte response Cases with achlorhydria tend to have a slightly smaller response and to manufacture blood more slowly than comparable cases with free hydrochloric acid in their stomach contents

The character of the curves yielded from plotting data obtained from daily reticulocyte counts in response to iron tend to differ somewhat from those obtained for pernicious anemia in response to liver or potent substitutes

SPINAL FLUID Denis Ayer Method for Estimation of Protein in, Ayers, J B, Dailey, M E, and Fremont Smith, F Arch Neurol & Psychiat 26 1038, 1931

The following modifications of their original method are described

METHOD

Into a test tube 0.6 cc of spinal fluid is measured To this are added 0.4 cc of distilled water and 1 cc of a 5 per cent solution of sulphosalicylic acid The contents of the tube are then mixed by inversion (but not by violent shaking) and, after being allowed to stand at least five minutes, are read against a standard protein suspension prepared at the same time as the unknown The standard is made by adding to a test tube 3 cc of a solution containing 30 mg of protein per hundred cubic centimeters and 3 cc of a 5 per cent solution of sulphosalicylic acid

Standard Twenty cubic centimeters of normal human blood serum is diluted to 200 cc with a 15 per cent solution of sodium chloride in a volumetric flask and filtered. This filtrate is the concentrated standard.

The total nitrogen of this filtrate is determined by the micro Kjeldahl method with 40 cc. The nonprotein nitrogen is determined in the original undiluted serum by the micro Kjeldahl method of Folin and this figure divided by ten is subtracted from the total nitrogen to obtain the protein nitrogen. The protein nitrogen multiplied by 6.25 gives the protein content of the concentrated standard.

The concentrated standard is diluted with distilled water to make the dilute standard containing 30 mg per hundred cubic centimeters.

The standards are preserved with a few crystals of thymol and kept on ice except when in use. In this way the authors have kept the concentrated standards for more than six months and the dilute standards for more than twelve months without appreciable change in the protein content.

Calculation

$$\frac{\text{Reading of the Standard } 30 \text{ (mg/100 cc in standard)}}{\text{Reading of the Unknown (cc of spinal fluid used)}} \times \frac{400}{400} = \text{mg protein per 100 cc}$$

With the standard set at 8, and with the use of 0.6 cc of spinal fluid, this is simplified to

$$\frac{400}{\text{Reading of the Unknown}} = \text{mg protein per 100 cc}$$

It is convenient to construct a table so that the protein values may be read off at a glance once the colorimeter reading has been made.

The authors consider the following as normal values: ventricular fluid, from 5 to 15 mg per hundred cubic centimeters, cisternal fluid, from 15 to 30 mg, and lumbar fluid, from 20 to 45 mg. Very rarely, they have found higher protein in the lumbar fluid, up to from 60 to 70 mg per hundred cubic centimeters, for which no cause could be found. It is possible that in such cases there was a pathologic process of the central nervous system which was unrecognized clinically, or that normal persons may occasionally have more protein than 45 mg per hundred cubic centimeters in the spinal fluid.

TUBERCULOSIS Tubercle Bacilli in the Blood Stream of Rabbits During the Course of Infection, Mishulow, L., and Park, W. H. J. Prevent Med 6: 95, 1932

From two experiments it seems probable that there is rapid localization of the tubercle bacilli inoculated into the blood stream, as shown by the tremendous decrease in their number between twelve and twenty-four hours after inoculation, and the steady decrease up to the fourth day.

The organisms persisted in the blood stream throughout the entire course of the infection, although they fluctuated in number from day to day. There was a marked rise in numbers on the day of death. This would justify the conclusion that there is a steady dissemination of the tubercle bacilli from the local lesion into the blood stream.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor,
Dr Warren L. Vaughan, Professional Building, Richmond, Va

The Human Factor in Industry

THIS is an interesting little booklet on the effect of fatigue, monotony, and environmental factors such as noise, crowding light, and ventilation upon output in industry.

The writer finds that there is a diminution in the amount of work done from hour to hour through the day and from day to day through the week. Longer work hours increase the frequency of accidents. When workers are at labor which interests them and which they like they produce more than when working on something that is distasteful. Some workers appear to do better work when they are following their normal rhythm. Machines worked with a central motor, in other words always at the same rhythm, at which several persons were working, turned out a certain amount of finished material. When this central motor was replaced by individual small motors, one for each worker so that each laborer could proceed at his work at the rhythm which best fitted him, the output was increased.

Frequent rest pauses in labor increase the total output. The duration of the rest varies with the type of industry. In a steel corporation men were loading ashes, coal and ore with the same type of shovel. Naturally, when ore was loaded with the same size shovel that was used for loading ashes, fatigue developed much more rapidly. When it was established that the size of the shovel should be varied depending on what is being loaded so that the weight lifted per shovelful would be the same whether the contents be ashes or ore, the results were much better. As a consequence the average amount shoveled per man per day rose from 16 to 59 tons and the staff was reduced from 500 to 150 men.

These and many other most interesting observations appear in the booklet which is the outcome of two series of lectures given in the Engineering Department of Glasgow University.

Chemical Methods in Clinical Medicine†

THIS book fills rather a unique place. It is not an ordinary textbook of clinical pathology nor is it purely a treatise on biochemical methods. It stands rather halfway between the two. All of the methods described are clinically practical methods but on the whole they are the less used of the clinical procedures. The volume is in essence a reference work in which one may find the practical methods for making the out of the usual chemical clinical laboratory studies. For this purpose it should find its way onto the shelves of all clinical pathologists for reference study. While all clinical chemical studies are discussed, especial attention is given to the chemistry of carbohydrate metabolism and the volume should be called especially to the attention of those interested in the study and treatment of diabetes. Scattered through the book one will find descriptions of numerous little technical laboratory tricks which facilitate routine work.

*The Human Factor in Industry. By E. P. Cathcart. CBE MD FRS. Gardiner Professor of Chemical Physiology University of Glasgow. Cloth. Pages 105. Oxford University Press American Branch New York 1925.
†Chemical Methods in Clinical Medicine. Their Application and Interpretation. With the Technique of the Simple Tests. By G. A. Harrison. B.A. MD BCh (Cantab) MRCS (Eng) LRCP (Lond). Reader in Chemical Pathology in the University of London. Reader and Lecturer on Chemical Pathology in St Bartholomew's Medical College. Chemical Pathologist to St Bartholomew's Hospital. With 2 colour plates and 63 illustrations. Cloth. Pages 534. The Macmillan Company New York 1930.

The Factor of Infection in the Rheumatic State

THIS is a monograph that will interest those who are making special study of acute and chronic rheumatism. The author develops his thesis by a unique method rarely employed in technical medicine but which maintains the interest and sequence most satisfactorily.

From his study the writer concludes that the rheumatic process is a reaction peculiar to susceptible individuals and representing a special type of tissue response to chemical substances resulting from disease of the upper respiratory tract. The bulk of evidence appears to incriminate the hemolytic streptococcus as an important factor. There is some evidence of contagion. Immigrants upon their arrival in New York often experience the first attack of acute arthritis. A group of arthritic patients transported from New York to Porto Rico were relieved of their recurrent attacks of acute arthritis during their stay in the South but the condition reappeared upon their return to New York. The hemolytic streptococcus was found in cultures of their throats in New York but not in Porto Rico. Studies of nurses entering training indicated that those who developed acute rheumatism did so after becoming infected with hemolytic streptococcus.

Upper respiratory infections appeared to be a most important factor in the genesis of the rheumatic state. However, an inherent susceptibility within the individual appeared necessary.

Diet in Disease

THIS is a strictly modern presentation of the subject, based upon the author's experience on the wards, in the Out Patient Department and in the lecture room at Johns Hopkins. While diet tables are abundant enough, emphasis is placed upon the principles of dietetics in the hope that the reader will acquire sufficient insight to be able to intelligently apply the dietary regimen rather than to merely hand out stereotyped cards. Digitalis is requisite in certain forms of heart disease, but it must be intelligently administered and in varying dosage under different circumstances. The same applies to diet in diabetes and to protein restrictions in nephritis. There is no doubt that too little attention is usually paid to anything but the most general principles in the dietary treatment of many diseases and this book should be a distinct aid to those who wish to intelligently supervise the feeding of those of their patients who are suffering from specific diseases, and to vary the diet depending upon the actual needs of the moment.

Part I discusses nutritional requirements. Part II gives a very adequate description of different varieties of foods and Part III details the principles of treatment of specific diseases including deficiency diseases, food allergy, undernutrition, hyperthyroidism, obesity, arthritis, fever, anemia, nephritis, heart disease, diabetes, acidosis, gastrointestinal disease, lead poisoning, and pregnancy. An appendix describes several of the restrictions of the Jewish dietary and gives several useful recipes.

Laboratory Diagnosis†

THERE are several points of difference between this volume and the many others on the same subject that have appeared within recent years. Well over half of the volume is taken up in Part I which deals with a discussion of diseases of the various organs and systems,

*The Factor of Infection in the Rheumatic State. By ALVIN F. COBURN, M.D., Resident Physician of the Presbyterian Hospital in the City of New York. Cloth. Pages 288. Seven color plates. Abundant case material. Very fully illustrated. The Williams and Wilkins Company, Baltimore, 1931.

**Diet in Disease. By GEORGE A. HARROP, JR., M.D., Associate Professor of Medicine, Johns Hopkins University, Associate Physician, Johns Hopkins Hospital. With 50 tables, sample diets and food lists. Cloth. Pages 404. Philadelphia, P. Blakiston's Son & Co., Inc., 1930.

†A Textbook of Laboratory Diagnosis. With Clinical Applications for Practitioners and Students. By EDWIN D. OSGOOD, M.A., M.D., Assistant Professor of Medicine and Biochemistry, Director of Laboratories, University of Oregon School of Medicine, Portland, Oregon, and HOWARD D. HASKINS, M.D., Professor of Biochemistry, University of Oregon School of Medicine, Portland, Oregon. With 21 figures in the text and 6 colored plates. Cloth. Pages 475. P. Blakiston's Son & Co., Inc., Philadelphia, 1931.

and of what positive laboratory observations may be anticipated in these different conditions. Part II details the laboratory methods in use by the authors. Since Osgood and Haskins have contributed a number of very material improvements and simplifications to routine laboratory procedure, especially in the line of practical blood studies, this section constitutes a really valuable reference source.

A distinct innovation is the special index, by diseases, so arranged that the clinician may refer to a particular disease under study and find in the index what special laboratory investigations should be made in that condition. There is also a general index. There is no section on bacteriology or serology.

The volume should be of interest both to the clinician and to the laboratory worker.

Tumors of Bone

THIS book, published under the auspices of the *American Journal of Cancer*, has for its purpose the presentation of the subject of bone tumors in an orderly and systematic manner.

The book is based mainly upon studies of material from the Surgical, Pathological Laboratory of Johns Hopkins Hospital and contains two chapters by Dr. J. C. Bloodgood, two as a foreword to the text proper, and concerned with therapeutic measures. The introductory chapter on interpretations of clinical findings is by Dean Lewis.

The twenty-two chapters of malignant neoplasms affecting bone have been systematically arranged and classified (and, in some instances reclassified), and reflect a careful and extensive study, not only of a large amount of clinical material but of the literature as well. The views expressed may be taken as matured opinions based upon critical analysis and correlation of available data, and, to that extent, is authoritative and representing a consensus of present opinion.

The volume is typographically commendable and excellently and profusely illustrated.

It is obvious that the price is only possible through the support of the Chemical Foundation. The publication is timely and should prove exceedingly useful as a practical and valuable reference.

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EDITORIAL

The Leucocytic Reaction of D'Amato in the Study of Disease

THE phrase "hemoclastic shock" first introduced by Widal and his co-workers in connection with their studies of leucocytic reaction to the introduction of protein, has now an accepted place in medical terminology, even though the significance first ascribed to it has been considerably modified.

The crises hémoclastiques of Widal, as is well known, indicates the temporary leucopenia consequent upon the ingestion of protein (milk) which he believed to be a delicate index of hepatic insufficiency and while the reaction has not entirely fulfilled the early claims made for it, the test, at least, has called attention to the existence of the phenomenon itself and has led to the application of the principle to other studies which, from the reports which have gradually accumulated during the past ten or twelve years, appear to be of definite interest and, perhaps, of distinct value.

The concept of a specific hemoclastic crisis in response to the introduction of

a specific protein was first advanced by D'Amato in 1921¹ and later reported upon somewhat more fully by D'Amato and de Durante in connection with a study of its occurrence in tuberculosis.

The reaction is based upon the leucocytic reaction following the subcutaneous injection of a small amount of tuberculin (one millionth of a gram or less), and is conducted as follows:

A total leucocyte count is made upon the fasting patient and the tuberculin injected. Total leucocyte counts are repeated thirty minutes later and every thirty minutes for two or three hours.

According to D'Amato a reduction of 600-800 per c. mm. in the total leucocyte count constitutes a doubtful reaction, 900-1000 constitutes a definitely positive reaction, a reduction approximating 2000 is markedly positive, and 3000 or over very markedly positive.

The leucopenia consistent with a positive reaction persists for about three hours, is constant in the same individual as shown by a repetition of the test, did not occur after the injection of proteins other than tuberculin, and was encountered consistently in tuberculosis and was consistently absent in the absence of this disease.

D'Amato believes the reaction analogous in many ways to anaphylactic shock and superior to the tuberculin reactions as ordinarily applied, because it is equally delicate and, moreover, easily and safely applicable to those cases in which focal, general, or febrile reactions are undesirable.

The D'Amato reaction in tuberculosis has been subject to study by many Italian investigators who have largely substantiated the claims made for it, among such reports being those of De Bonis,³ Sanguigno,⁴ Romito,⁵ and Macchiaio.⁶

In Italy, therefore, the D'Amato reaction has been rather widely used and generally accepted as of specific diagnostic value in the study of tuberculosis.

It has also been applied in principle in the study of various infectious diseases among them typhoid fever, undulant fever, gonorrhea, pertussis, tinea, leishmaniasis, and glanders.

In typhoid fever and Malta (undulant) fever, the antigen used to provoke the reaction is a vaccine in a dose of twenty to thirty millions per cubic centimeter which is injected subcutaneously, a leucocyte count being made before and thirty minutes after the injection.

In these infections the reaction has been reported upon favorably by numerous workers.⁷⁻¹²

In these conditions it was noted that the reaction may be quite transient so that it may be necessary to repeat it, and also that it was nonspecific in paratyphoid infections, that is, that it occurred with either A or B vaccine regardless of whether the infecting organism was para A or para B.

There were indications, also, as noted by Gasparini, that some prognostic value might be attached to the procedure, as the reaction was usually marked in benign cases and less intense in severe cases. Testolin and Pujatti speak of the D'Amato reaction as giving "brilliant results" in typhoid fever, and found it of value also in pneumonia (lobar and lobular), streptococcus infections, and gonorrhea, especially in the female.

They cite, as an instance of the specificity of the reaction, a positive reaction after antityphoid vaccine in a case proved by autopsy to be military tuberculosis but in which the typhoid bacillus was isolated from the feces, and another positive reaction in cholecystitis in which the bile contained typhoid bacilli.

Equally good results are reported in whooping cough by Fanton,¹⁴ and in timea by Barboglia,¹⁵ in the latter condition the antigen being one cubic centimeter of a 1:20 dilution of trichophyton in distilled water. From a study of fifty cases he regards the reaction as quite specific, delicate, and reliable.

In leishmaniasis Gatto¹⁶ reports the reaction inconstant except in patients who had been intensively treated which he explains on the basis of destruction of the parasites by the treatment and subsequent sensitization by their protein.

Bonanno¹⁷ reports upon the reaction in echinococcus disease, and Bozzelli¹⁸ upon its occurrence in glanders, while it has also been studied in pregnancy by Cappellani,¹⁹ Lenzi,²¹ and Longo,²² and in tumors by Barbera,²³ Citelli and Carco,²⁴ and Bossa,²⁵ and in gonorrheal infections by Mossetti,²⁶ Santoranni,²⁷ and Aimanino and Bertolotti.²⁸

Perhaps the greatest interest, however, centers upon the studies made of D'Amato's reaction in syphilis, its application to the diagnosis of this disease being suggested by D'Amato⁹ in 1927.

The D'Amato reaction in syphilis is applied as follows. A total leucocyte count is made upon the fasting patient and 2 centigrams of bimiodide of mercury injected hypodermically. A second leucocyte count is then made within half an hour, not longer, as the reaction may be transitory. The interpretation of the reaction is the same as already described above.

The reaction also followed the intravenous injection of neotriopol and neosalvarsan, being well marked after the latter.

In none of the cases tested was any leucopenia produced by the injection of ordinary proteins.

D'Amato reports very consistent results. In 204 cases of known syphilis the reaction was positive in 180 or 88 per cent and doubtful in 16, persisting after the patient had become seronegative under treatment.

D'Amato's report has been followed by a number of others.¹⁰⁻¹⁴

In general, his results appear to be confirmed.

It would appear that the reaction is most consistent following the intramuscular injection of soluble preparations of mercury or bismuth and arsphenamine, and that it corresponds quite closely to serologic results.

Fanton²¹ found it especially useful in the study of hereditary and congenital syphilis but emphasizes that nonspecific positive reactions may be encountered in the presence of endocrine disturbances.

Goun, Bienvenne, and Peres²⁹ have recently described a variation of D'Amato's reaction as follows:

A leucocyte count is first made upon the fasting patient. An immediate injection of antisiphilitic medicament is then administered and a leucocyte count made two hours later.

A leucocytosis (increase of 1000 or more) constitutes a positive reaction.

According to Goun and his collaborators a leucocytosis indicates not only the presence of syphilis but also that it is reacting favorably to treatment while

a leucopenia signifies simply the absence of a reaction without distinguishing between syphilitic and nonsyphilitic cases

In a subsequent paper,¹⁰ two types of this reaction are distinguished

1 "Reaction of presence, which appears earlier than the serologic reactions and which Goun and his collaborators regard as diagnostic of syphilis

2 "Reaction of defense," in which a negative reaction encountered in known syphilis indicates that the drug in question will not be efficacious in treatment

The difference between the reaction described by D'Amato and that described by Goun appears to be largely one of interpretation of the results

Both reactions have been studied simultaneously by De Blasio,¹¹ and by Vaiga.¹² The results of both investigators are in accord and may thus be summarized

While, until further work has been done it is impossible to pass any final opinion, this much seems fairly well established

1 Neither the hemoclastic reaction of D'Amato nor the variant described by Goun are as valuable as the serologic reactions because they are neither as delicate, as constant, nor as specific

2 Both reactions require extremely careful attention to detail to eliminate fallacious results due to technical errors

3 Nonspecific reactions may occur with both methods

4 D'Amato's reaction is more reliable than that of Goun

In the last analysis it must be emphasized that the leucocyte reaction in syphilis must be regarded as only a relative sign of the disease and must be correlated carefully with all the other evidence pro and con

Its most apparent and distinct value is as a means of directing attention toward the *possibility* of syphilis in a particular case, a possibility which must be determined or eliminated by other and more conclusive methods

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THE VALUE OF AQUEOUS EQUINE LIVER EXTRACT, GLYCERATED IRON, AND HEMOGLOBIN IN THE TREATMENT OF SECONDARY ANEMIAS*

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WE HAVE recently reported^{1, 2} on the presence of the "antipernicious anemia" principle in the liver of the horse. Both the oral and subcutaneous equine liver extracts containing the active principle soluble in 70 per cent alcohol, described by Cohn³ in the preparation of fraction G, produced prompt hematologic remissions when administered to pernicious anemia patients. The benefit of this fraction in the treatment of anemias of the pernicious type, including sprue, fish tapeworm infestations, certain cryptogenic hyperchromatic anemias, and chronic anemias of long standing dietary deficiency diseases, has been established. Giffin's⁴ suggestion that liver extract may be beneficial in the treatment of anemias associated with organic gastric disease was not corroborated by Castle's⁵⁻⁷ recent work. These investigators were able to demonstrate that the activating substance absent in the gastric secretion of patients with pernicious anemia, was present in four patients with anemia having a total anaclidity, and absent in two patients with pernicious anemia-like blood pictures having an apparently normal gastric juice, the former responded to iron therapy, the latter responded to liver extract therapy.

It is generally recognized that the 70 per cent alcohol soluble fraction of liver is not effective or only mildly effective in the treatment of secondary anemia, in contrast to the frequent good responses obtained from the feeding of whole liver. Robschert-Robbins⁸ demonstrated in experimental posthemorrhagic anemias that the alcohol soluble liver extract only retained 15 per cent of the potency of whole

*This work was made possible by a grant from the Chappel Foundation for Organotherapeutic Research and was done at Northwestern University and Cook County Hospital.

liver. Futh and Singer⁴ were unable to obtain any effect on experimentally induced anemias with Minot's liver extract, and found the regeneration period definitely shortened when whole liver was added. Earlier work by Whipple¹⁰ showed that active substances were found in watery extracts, alcoholic extracts and in the extracted liver residue. The sum of these three sources of regenerating power obtained from experimental anemic dogs was from sixty to seventy grams of hemoglobin over a two weeks' period. The inorganic ash of beef liver was likewise potent in their experimental anemias, but it only contained one-half the potent factors present in whole liver—Robschert-Robbins.¹¹ This led to the conclusion that a group of substances present in whole liver were responsible for the increased hemoglobin response in experimental anemias. Following this Whipple¹² obtained a fraction assaying 70 per cent of the potency of whole liver in producing hemoglobin regeneration on posthemorrhagic anemic dogs. The summary of the clinical reports shows that whole liver is more beneficial in the treatment of secondary anemias than Minot's extract (Middleton¹³ Dyke,¹⁴ Powers,¹⁵ Keefer¹⁶).

Minot and Murphy¹⁷ concluded from their observations that liver contained a substance which was specific in the transformation of a megaloblastic bone marrow to a normoblastic one. The active principle in liver as yet has not been isolated, although certain characteristics and properties of the antianemic material have been determined. Cohn, et al.,¹⁸ presumed from their chemical refractionation of the effective material that the active substance is in the nature of a nitrogenous base or polypeptid.

Robschert-Robbins and Whipple¹⁹ demonstrate in their earlier work that whole liver plus non salts was more beneficial in the treatment of posthemorrhagic anemia than either substance when administered alone. On the combined feeding they were able to produce as high as 140 grams of hemoglobin per two weeks' period in their experimental anemic dogs. Keeter and Yang¹⁶ from their clinical report of 37 cases of secondary anemia concluded that whole liver and non given in combination were more effective than either given alone. This was further substantiated by the clinical reports of other investigators.

The value of inorganic elements other than non in the treatment of anemias has received widespread interest. Hart, Steenbock, and their coworkers²⁰ demonstrated regeneration of hemoglobin by the addition of small amounts of copper to nutritional anemic rats which were on minimal amounts of pure non, inadequate to produce hemoglobin regeneration. Elvehjem, et al.,²¹ have recently shown that a trace of copper is necessary as a catalyst for inorganic non in the construction of the hemoglobin molecule. Satisfactory clinical results were reported by Lewis,²² Josephs,²³ and Mills,²⁴ on the administration of non and copper in the treatment of nutritional and idiopathic anemia in infancy, childhood, and adults. The summary of the current literature as to the value of germanium, arsenic, manganese, nickel, cobalt, and other inorganic elements in the regeneration of hemoglobin leaves the matter still in doubt.

Our work was undertaken to determine the value of a mixture of an aqueous concentrated liver extract, neutral glycerol-non, and blood, in the treatment of secondary anemias. Preliminary studies in posthemorrhagic anemic dogs gave promising results.

THEORY AND METHODS

The liver fraction used was an aqueous extract from fresh horse livers, unpurified and evaporated in vacuo, so that one part of the extract represented four parts of liver. The fraction active in secondary anemia, as described by Whipple¹⁴ was present in this extract. One of us, Meyer,¹⁵ was able to demonstrate greater hemoglobin regeneration on the administration of this aqueous concentrated liver extract to posthemorrhagic anemic dogs than the equivalent amount of the 70 per cent alcohol soluble liver fraction containing the active "antipeptic anemia" principle.

Ferrous chloride was added to the above concentrated aqueous liver extract as a neutralized glycerol solution which was incorporated in defibrinated blood serum. It may be concluded from the reports of Starkenstein¹⁶ and Remann and Fritsch¹⁷ that ferric compounds are less readily absorbed than the bivalent iron. The soluble iron salts are transformed into chlorides in the stomach, and partially into hydroxides or carbonates when coming in contact with the alkaline pancreatic juice, and in this way their absorption is hindered. It was thought that a mixture of ferrous chloride and glycerol might be of greater value than ferrous chloride alone since glycerol prevents the precipitation of iron by alkali.

In the preparation of this compound, a concentrated solution of ferrous chloride was mixed with an excess of glycerol and the reaction of the mixture was adjusted to P_H 6.8. The object of this partial neutralization was to make it less astringent to the mucosa of the stomach. This neutralized glycerol-iron solution is not perfectly stable when diluted with water. A precipitate is apt to be formed on longer standing. However, it was found that serum acts as a protective stabilizing colloid, and that the presence of the formed blood elements does not interfere with this effect. In using defibrinated blood for this purpose, one also introduces the possible additional benefit of hemoglobin. In order to determine the relative effect on hemoglobin regeneration of this neutral glycerol-iron compound, equivalent amounts of iron solutions in the form of aqueous ferrous chlorides and acid glycerol iron were administered to "control" groups of experimental anemic dogs. Although comparatively good responses were obtained in all three groups, greater and more consistent hemoglobin regeneration was noted in the group on the neutralized glycerol-iron compound.²⁰

The ineffectiveness of hemoglobin in anemic animals found by Abderhalden²¹ was probably due to the rather low iron content (336 per cent), which was evidently insufficient to produce hemoglobin regeneration. It seems, therefore, that the administration of blood in large enough quantities to supply the desired amount of iron is impractical. Even though the effective value of hemoglobin as a source of iron is limited, the organic component might play a certain rôle. It is obvious that the pyrol complex of porphyrin may be formed in different ways if it should not be present in the food in sufficient quantities. But just as the assimilation of iron can be impaired, it is possible that the synthesis of porphyrin may not be performed satisfactorily in certain anemias. In such cases of anemia the porphyrin part of hemoglobin may be just as important as the iron. An addition of inorganic iron in a suitable form may be of benefit in making the hemoglobin more effective.

Theoretically, a combination of concentrated aqueous liver extract and the neutralized glycerol-non compound in defibrinated blood includes all the possible known factors concerned in hemoglobin regeneration. The posthemorrhagic anemic dogs receiving this combined mixture showed a greater and more uniform erythrocyte and hemoglobin response than the groups on the various individual components.²⁰

Syrup and flavoring agents were added to improve the taste and alcohol as a preservative (14½ per cent). This mixture is designated as preparation Bl 105 in this report. A careful analysis of one and one-half ounces of this preparation, which was the daily average dose administered to our cases, shows that it contains the extract of 84.4 grams of whole liver, a total of 104.24 mg of metallic iron (6.75 mg from liver, 5.62 mg from hemoglobin, and 91.87 mg from the neutral glycerol-non compound) and a total of 1.4 mg of metallic copper.

A comparative study was made by treating a series of patients with secondary anemia as "controls" on a known "weak" preparation, Bl 101. One and one-half ounces of this preparation, which was the amount administered daily, contained liver broth expressed from 16.8 grams of boiled liver, 10.6 mg of iron, and traces of copper, to which the same amounts of flavoring agents and alcohol was added as in Bl 105.

The clinical work was made possible through the courtesy and cooperation of the Cook County Hospital Staff. The cases reported were patients with secondary anemias of the common variety occurring in a large institutional practice. No attempts were made in selecting ideal cases. The patients were accepted routinely as they entered the hospital, irrespective of the type or seriousness of their malady. Proper medical and surgical care was immediately instituted, whenever necessary. Most of these patients received a well-balanced diet, with the exception of a few patients who required special dietary management. Complete blood examinations including reticulocyte counts were made at one- to five day intervals during the period of hospitalization, and at two-week intervals in a follow-up clinic when discharged.

It is obvious that in most instances individual study is necessary, as each patient presents a complex picture of various causative factors for the anemia, which is seldom identical in two patients with the same disease. For this reason the types of secondary anemias under observation in the "treated" groups were only classified according to their "chief" etiological causes. A total of 112 cases have been studied.

RESULTS

The first twenty-nine patients under observation with secondary anemia were given one and one-half ounces of the known "weak" preparation (Bl 101) daily. Because of the limited number and diversity of cases in this series, the results obtained were tabulated under one group, Table I. Moderate improvement was observed in Cases 4, 8, and 20, which was undoubtedly due to the usual hematopoietic response following acute hemorrhages. It is quite evident from the summary of this series, showing an average daily gain of 0.34 per cent hemoglobin and 2,500 red blood cells, that the hematologic response was only slightly influenced by the "weak" preparation (Bl 101) or by the specific surgical or

medical therapy during the period of observation. Because little or no progress was made on this preparation by Patients 10 and 20 to 29 (inclusive), the treatment was changed to the more potent preparation Bl 105 following which, with the exception of Cases 26 and 28, the hemoglobin and the red blood cell count became normal. Case 26, an inoperable gastric carcinoma, showed a moderate response, while Case 28, a bacterial endocarditis, showed no response to either preparation.

The results obtained from the administration of one and one-half ounces daily of Bl 105, the concentrated liver-iron and hemoglobin mixture, to patients with various types of secondary anemia, are recorded in Tables II, III, IV, IV-A, V, and VI.

The cases classified under Table II were of the acute hemorrhagic type and include diseases associated with frank hemorrhage which was the predominating cause of the anemia. The diseases primarily responsible for the acute anemia in this group consisted of incomplete abortions, ruptured ectopic pregnancies, placenta previa, a postpartum hemorrhage, and a bleeding peptic ulcer. It is obvious that stopping the source of hemorrhage is the most important factor in recovery. It also seems logical that the administration of a concentrated preparation supplying in an easily assimilated form the necessary blood-building elements plus the addition of a hemopoietic stimulant may shorten the period of blood regeneration and convalescence. Treatment was started as soon as permissible, each patient receiving one-half ounce of preparation Bl 105 three times a day. In many instances moderate bleeding continued for several weeks while on therapy. The best gains were observed in this series, showing an average daily gain of 537 per cent hemoglobin and 30,538 red blood cells. The additional increase is probably explainable by the acute short hemorrhages being insufficient to deplete the body of the blood-building elements and the good response of the preexisting healthy bone marrow.

Patients with histories of moderate or profuse bleeding, the duration of which was from one month to several years, were classified under Table III as chronic posthemorrhagic anemias. The diseases most commonly met with in these cases were bleeding fibroids, bleeding hemorrhoids, and bleeding peptic ulcers. In most instances surgical treatment was necessary to eradicate the source of hemorrhage, with the exception of the patients with bleeding peptic ulcers, who were placed on Sippy's management. Each patient received one-half ounce of the more concentrated whole-liver-iron preparation Bl 105 t i d, which was instituted shortly after their admittance to the hospital. Of the twenty-nine patients treated in his series, twenty-three, with an initial average of 40 per cent hemoglobin and 2,420,000 red blood cells per cubic millimeter, became normal during an average period of 75.3 days, with an average daily response of 541 per cent hemoglobin and 24,435 red blood cells.

Although the results in this series of patients resemble those obtained in the anemias which followed acute hemorrhages, the somewhat smaller response can probably be attributed to the long period of continuous bleeding with the resultant depletion of the blood-building elements, and an overstimulated and fatigued hematopoietic system. Moderate continuous bleeding persisted in several patients, ten in this series, while on treatment, and, although a normal hemo-

TABLE I

CASES OF SECONDARY ANEMIA FROM VARIOUS CAUSES TREATED ON KNOWN WEAK PREPARATION BI 101 AS "CONTROL" 1/2 OUNCE T I D

CASE	SEX	AGE	DIAGNOSIS	TOLERABLE DURATION	DAYS ON TREATMENT	CHANGES IN				WEIGHT CHANGES POUNDS	COMMENTS
						HEMOGLOBIN SAHLI (%) BEFORE	HEMOGLOBIN SAHLI (%) AFTER	RBC IN MILLIONS BEFORE	RBC IN MILLIONS AFTER		
1	F	29	Incomp abortion Congenital heart	15 da	28	45	38	2.01	2.16		No prog*
2	F	41	Bleed fibroids	1 yr	24	34	40	2.78	3.07		Hysterectomy*
3	M	30	Osteomyelitis of elbow joint	2 mo	46	52	58	3.07	3.41	120 145	Dr mgc*
4	F	28	Incomp abortion	1 mo	35	41	47	1.82	2.31	107 109	Curettage*
5	M	31	Bact endocarditis	6 mo	36	37	27	2.45	1.78		Progressively downhill Went home
6	F	27	Incomp abortion	11 da	30	39	39	2.94	2.74	119 123	Placenta removed
7	F	34	Salpingitis Pelvic peritonitis	2 1/2 yr	55	53	58	3.93	3.26		Bilateral salpingectomy continued several weeks operative*
8	M	60	Bleed gastric ulcer	10 da	20	57	69	2.60	3.88		Also on supply management Transferred from hospital
9	F	30	Postoperative thrombophlebitis	10 da	17	29	36	2.04	3.14		Low grade temp throughout*
10#	F	29	Incomp septic abortion	1 wk	69	46	45	2.10	3.12		Placed on BI 105 Normal in 91 da
11	M	47	Bruits disease	1 yr	57	52	61	2.52	3.03	143 150	Table IV A, Case 10
12	F	23	Septic abortion	4 1/2 mo	34	38	48	2.50	2.37		*
13	M	35	Empyema	10 da	27	64	63	3.14	3.78		Rib resection Dr mgc*
14	F	20	Incomp septic abortion	3 da	16	40	53	2.73	3.20		Had 150 cc blood transfusion 2 days prior*
15	F	26	Bilateral salpingitis Pelvic peritonitis	6 wk	34	68	45	2.79	2.77	131 131	Bilateral salpingectomy*
16	M	15	Encapsulated empyema	4 wk	33	30	43	2.59	2.31		Rib resection Dr mgc*
17	F	38	Comp abortion	3 wk	68	45	51	2.40	3.39	147 146	Curettage*

TABLE I—CONT'D

CASE	SEX	AGE	DIAGNOSIS	PROBABLE DURATION	DAYS ON TREATMENT	CHANGES IN				WEIGHT CHANGES POUNDS	COMMENTS
						HEMOGLOBIN SALES (%)		RBC IN MILLIONS			
						BEFORE	AFTER	BEFORE	AFTER		
18	M	13	Septic 2nd deg burns	2 mo	67	64	62	167	3.09	171 174½	* Salpingectomy 400 cc blood given 3 da prior to treatment Changed to BI 105 Table II, Case 1, Comp Table IV, Case 3, Comp
19	F	36	Ectopic preg ruptured	4 da	14	52	47	292	2.62		
20#	F	21	Ruptured ectopic preg	1½ da	49	36	65	219	2.72		
21#	F	28	Pelvic peritonitis Thrombo phlebitis following hysterectomy	5 mo	67	60	65	355	3.60		
22#	F	19	Rheumatic heart disease	3 wk	67	29	43	295	3.38	109 115	Table IV, Case 4, Comp Table IV, Case 7, Comp
23#	M	67	Decompensated heart Idio pathic anemia	4 yr	16	25	28	181	2.01		
24#	F	40	Bleed fibroids	2 yr	63	34	37	309	3.12		Hysterectomy Table III, Case 9, Comp
25#	F	43	Hydatiform mole	3 mo	44	39	41	213	2.96		Hysterectomy Table III, Case 10, Comp Table V, Case 7
26#	M	63	Cn of stomach	2 yr	79	31	19	110	1.73		Hysterectomy Table III, Case 13, Comp Table IV, Case 16, Incomp Table IV, Case 2, Comp
27#	F	39	Bleed fibroids	2½ mo	25	49	56	342	2.79		
28#	F	44	Breast endocarditis	1 yr	65	42	44	283	2.28		
29#	F	27	Purpura hemorrhagica	4 yr	92	62	73	419	3.60		
Total					1276	1301	1348	8035	83.66		
Average Patient					44	44.9	46.4	277	2.88		
Average Net Gain						15		0.11			
Average Daily Gain						0.34% Hb (Salih)		2500 RBC			

F Indicates female M Indicates male *Cases that left hospital and did not return for further observation † Changed to BI 105

SECONDARY TABLE II

RESULTS OBTAINED IN CASES OF SECONDARY ANEMIA OF THE ACUTE POSTHEMORRHAGIC TYPE TREATED WITH PREPARATION BL 105 1 OUNCE TID (RECOVERY COMPLETE)

CASE	SEX	AGE	DIAGNOSIS	PROBABLE DURATION DAYS	DAYS ON TREAT- MENT	CHANGES IN				WEIGHT CHANGES POUNDS	COMMENTS
						HEMOGLOBIN SAFILI (%)	BEFORE	AFTER	RBC IN MILLIONS		
1	F	21	Ectopic pregnancy	14	60	65	272	86	4.35	99 113½	Salpingectomy†
2	F	29	Incomp abortion	12	47	28	28	80	4.00	147 151	Curettage
3	F	40	Incomp abortion	21	88	62	276	82	1.23	107 118	Placenta expressed
4	F	20	Retained placenta	14	106	51	243	81	4.25	99 100	Curettage # 1
5	F	26	Ruptured ectopic pregnancy	1	44	58	270	84	4.18	145 150	Salpingectomy
6	F	38	Ruptured ectopic pregnancy	4	44	50	230	81	4.11	199 199	Salpingectomy
7	M	49	Bleeding peptic ulcer	21	63	31	1.99	81	4.18	155 184	Gastroenterostomy
8	F	29	Incomp abortion	1	94	57	2.90	86	4.95	139 135	Curettage
9	F	27	Incomp abortion	1	12	17	2.94	83	4.53	101 104	Curettage # 2
10	F	27	Ectopic pregnancy	1	41	39	1.92	81	4.82	113 115	Salpingectomy # 3
11	F	21	Incomp abortion	32	83	36	75	75	4.08	149 150	Expelled placenta
12	F	36	Incomp abortion	22	87	25	83	83	1.29	120 132	Also had rectal bleeding
Total						552	27.05	983	51.42		
Average Patient						16	2.25	81.9	4.29		
Average Net Gain						35.9			2.04		
Average Daily Gain						0.547% Hb (Saiba)			30,538 RBC		

*SIMILAR CASES, SEEN ON TREATMENT (NOT COMPLETED)

13	F	24	Incomp abortion	20	17	19	1.61	60	3.46		Expelled placenta
14	F	26	Postpart hemorrhage	3	86	18	0.91	77	1.05		Moderate temperature through- out treatment # 1
15	F	25	Postpart hemorrhage	3	46	53	2.59	70	3.88		Moderate temperature
16	F	28	Placenta previa	3	29	31	1.38	70	3.42		Also had breast abscess
17	F	30	Placenta previa	3	29	42	3.25	53	3.25		Labor terminated
18	F	35	Incomp abortion	14	16	18	2.08	25	1.19		Curettage
19	F	33	Incomp abortion	7	37	40	2.26	71	3.93		Moderate temperature
20	F	26	Incomp abortion	7	16	24	1.66	45	3.17		Placenta expelled

*Average daily Hb and RBC gain calculated on complete cases only
 F indicates female M indicates male

†No response in 19 days to BL 101 # 1 100 cc blood given 2 wks prior to treatment # 2 500 cc blood given 10 days prior to treatment # 3 150 cc blood transfusion 2 days prior to treatment # 4 540 cc blood transfusion at beginning of treatment

globin and red blood cell count was produced, this was also probably a contributing factor in producing a lower average daily gain. Cases 9, 10, and 13 (Table III) previously treated with the "weak" preparation (Bl 101) and showing little progress, became normal on treatment with the more concentrated preparation (Bl 105).

The secondary anemias in Table IV treated with preparation Bl 105 showed an average daily gain of 3 per cent hemoglobin and 16,400 red blood cells in the completed series, and 22 per cent hemoglobin and 10,700 red blood cells in the incompleting group. Ten of the twenty-four patients in this group became normal on treatment during an average of 101.6 days. Patients who failed to return to the clinic, and those who are still under observation, are classified in the incompleting group. Many etiologic and contributing factors entered into the production of the anemia in this group, which included patients primarily with infectious, toxic, and dietary diseases.

Very little response was obtained in Cases 13, 16, 17, 23, and 20 of the incompleting group, which consisted of a terminal glomerular nephritis, a bacterial endocarditis, two cases of far-advanced tuberculosis, and one of Hodgkin's disease running a septic course. Although slow progress was made in the cases with low-grade septic processes, the hemoglobin and erythrocyte count became normal after long-continued treatment on preparation Bl 105. Case 7 in Table IV, a young female with a congenital splenic anemia, gave a history of eating liver and taking liver extracts for the past five years, and, although moderate improvement was noted, she at no time felt well enough to carry on her daily activities. This patient was started on preparation Bl 105 following splenectomy. The splenectomy was probably the fundamental factor in her complete recovery, although it was interesting to note that there was a moderate drop in the hemoglobin and the erythrocyte count dropped when taken off of treatment during an interval of 49 days (Table VI, Case 22).

The patients classified under Table IV-A, treated with whole-liver-iron preparation, showed an average daily gain of 43.4 per cent hemoglobin (Sahli) and 18,300 red blood cells. We believe that the data obtained from these eleven cases constitutes the best evidence that the concentrated liver-iron preparation (Bl 105) was really effective in improving the anemia. In these cases we are of the opinion that the improvement may only be accounted for as being the result of the whole-liver-iron therapy. Although we believe that a number of the other patients recorded in the tables were benefited by the concentrated liver-iron preparation, their improvement may be accounted for in other ways.

It is interesting to note the good response of Cases 1 and 6 to preparation Bl 105. Both patients were white women, about forty years of age, giving a history of long continuous rectal bleeding which came on only before and during each menstrual period. A long dietary deficiency history was also obtained in both instances. Their chief complaints were weakness, pallor, loss of appetite, and occasional gastrointestinal disturbances. Careful gastrointestinal study and numerous proctoscopic examinations failed to reveal the source of bleeding, which continued throughout the entire course of treatment. The essential findings in both patients were an achylia gastrica and a high-grade secondary anemia, with a low-color index and leucopenia, of the chronic "chlorotic" type first described by

SECONDARY TABLE III

RESULTS OBTAINED IN CASES OF SECONDARY ANEMIAS OF THE CHRONIC POSTHEMORRHAGIC TYPE TREATED WITH PREPARATION Bt 105 1 OUNCE TID (RECOVERY COMPLETE)

CASE	SEX	AGE	DIAGNOSIS	PROBABLE DURATION	DAYS ON TREATMENT	CHANGES IN				WEIGHT CHANGES POUNDS	COMMENTS
						HEMOGLOBIN SAMPLE	BEFORE	AFTER	BC IN MILLIONS		
						BEFORE			AFTER		
1	M	54	Hypertension and bleeding peptic ulcer	30 da	74	42	72	177	355	131 115	Also on Sippy treatment
2	F	29	Misc placenta previa	12 da	19	33	89	167	440	151 160	Labor terminated by bag induction
3	F	38	Bleeding fibroids	2 yr	118	15	80	261	124	142 162	Hysterectomy
4	F	36	Bleeding fibroids	1 yr	90	41	81	220	465	120 132	Hysterectomy
5	F	38	Bleeding fibroids and pregnancy	7 mo	125	59	80	140	124	125 136	First operation exploratory. Pig removed, second hysterectomy
6	F	44	Bleeding fibroids	1 yr	77	42	91	172	424	155 160	12 x-ray treatments 1 yr ago, pain hysterectomy prior to med
7	M	49	Bleeding peptic ulcer	1 mo	69	27	55	204	320	148 163	Also on Sippy management
8	F	43	Bleeding fibroids	3 yr	55	60	95	278	111	126 115	not return to hospital
9	F	40	Bleeding fibroids	2 yr	73	37	82	112	425	122 122	Hysterectomy #1
10	F	43	Histiocytoma mole	3 mo	95	41	85	296	112	95 96	Hysterectomy followed by large stitch abscess
11	M	40	Bleeding hemorrhoids	6 mo	60	14	81	115	158	189 206	Hysterectomy, 4 x-ray treatments while on treatment
12	F	31	Incomp abortion	3 mo	59	57	82	283	113	111 110	Hemorrhoidectomy
13	F	49	Bleeding fibroids and old double mitral lesion	6 mo	71	56	83	279	137	118 120	Curtailed
14	M	36	Bleeding hemorrhoids, double mitral lesion	13 yr	77	16	90	110	156	117 111	Hysterectomy followed by stitch abscess
15	F	32	Bleeding fibroids	7 mo	56	38	84	261	471	107 110	Hemorrhoidectomy
16	M	27	Bleeding hemorrhoids	7 mo	51	18	83	356	491	160 161	Hysterectomy #2
17	M	13	Bleeding hemorrhoids	4 yr	67	41	80	257	119	165 171	Hemorrhoidectomy

SECONDARY TABLE III—CONT'D

CASE	SEX	AGE	DIAGNOSIS	DURATION	DAYS ON TREATMENT	CHANGES IN				WEIGHT CHANGES POUNDS	COMMENTS
						HEMOGLOBIN SAULT (%)		RBC IN MILLIONS			
						BEFORE	AFTER	BEFORE	AFTER		
18	F	46	Fibroid uterus	1 yr	114	37	50	1.00	1.06	143 163	Hysterectomy
19	F	36	Incomp abortion	3 mo	58	45	77	2.18	4.10	129 134	Spelled placenta in hospital
20	F	31	Bleeding fibroids	1 yr	98	20	86	1.10	4.30	96 99	Hysterectomy preceded by radium treatment
21	M	45	Bleeding peptic ulcer	5 mo	112	25	80	1.15	4.29	130 145	Also on Sippy management Mod bleeding during first half of treatment
22	F	32	Incomp abortion	4 mo	63	28	82	1.15	4.10	126 127	Curative
23	M	38	Bleeding peptic ulcer	6 wk	29	59	83	2.97	4.23	152 155	Also on Sippy management
Total						930	1877	55.56	98.46		
Average Patient						40.8	81.6	2.42	4.26		
Average Net Gain						40.8		1.84			
Average Daily Gain						0.541% Hb (Sault)		24.435 RBC			

*SIMILAR CASES STILL ON TREATMENT (NOT COMPLETED)

24	F	47	Bleeding fibroids	6 mo	72	23	74	1.67	3.97	187 195	Hysterectomy
25	F	31	Bleeding fibroids	1 mo	38	37	57	2.11	3.73		Hysterectomy
26	M	38	Bleeding hemorrhoids	2 yr	72	29	61	2.67	3.75	102 204	Hemorrhoidectomy # 3
27	F	31	Bleeding fibroids	5 yr	57	29	70	1.91	3.93	163 175	Hysterectomy # 3
28	M	31	Spondylitis	1 yr	38	30	59	2.58	4.31		Mod bleeding while on treatment
			ulcer and old mitral insuff								Also on Sippy management
29	F	36	Bleeding peptic ulcer	1 mo	43	38	56	2.76	4.06		Also on Sippy management

* Average daily Hb and RBC Gain calculated on complete cases only. F indicates female. M indicates male. # 1 Two 500 cc blood transfusions given on 2 months prior to medication. † On Bl 101 for 2 months. No progress. ‡ No progress on Bl 101 in 14 days. § 250 cc blood transfusion given prior to treatment. # 3 Did not return for further treatment. # 4 Made no progress in 3½ weeks prior to medication. † No progress on Bl 105 in 25 days.

TABLE IV

RESULTS OBTAINED IN CASES OF SECONDARY ANEMIAS RESULTING FROM INFECTIONS, TOXIC, AND DIETARY DISORDERS (RECOVERED COMPLETELY)
Treated with Preparation BI 105 $\frac{1}{2}$ Ounce T I D

CASE	SEX	AGE	DIAGNOSIS	PROBABLE DURATION	DAYS ON TREATMENT	CHANGES IN				WEIGHT CHANGES POUNDS	COMMENTS
						HEMOGLOBIN SAVER (%)	BEFORE	AFTER	RBC IN MILLIONS		
1	M	52	Poliagra Polynucleosis	6 mo	102	57	84	4 31	2 50	146 170	Nervous symp improved Able to carry on normal activities
2	F	27	Achondroplastic dwarf Anemia of pregnancy	5 mo	94	51	83	4 04	2 14	84 86	Cesarean section On treatment at end of preg and following surg
3	F	22	Incomplete septic abortion	1 mo	112	50	81	4 87	2 09	104 118	Curt tge. Febrile throughout therapy and profuse vaginal bleed
4	F	23	Incomplete septic abortion	3 mo	90	29	80	4 42	1 62	118 120	Curt tge
5	F	25	Preg subcutaneous abscess	4 wk	65	47	82	4 22	2 22	101 111	Abscess drained 1 mo prior to delivery when treat was started
6	F	37	Incomp abortion Generalized peritonitis pelvic abscess	3 mo	181	66	83	4 10	2 72	118 173	Colpotomy Septic course through out
7	F	18	Congenital splenic aneurysm	5 yr	57	59	83	4 53	3 58	134 127	Splenectomy 2 wk prior to therapy
8	F	39	Incomp septic abortion	7 da	189	43	83	4 14	2 23		Low grade temp throughout
9	F	42	Chronic infectious mononucleosis	3 yr	11	73	86	4 89	3 97		# 1
10	F	29	Incomp septic abortion	1 wk	91	46	81	4 31	3 12		No response to BI 101 in 69 da
Total						512	823	27 18	13 82		Table 1, Case 10
Average Patient						51 2	82 3	27 1	438		
Average Not Gained						41 1			1 66		
Average Daily Gain						0 3%	1 lb (Saver)		10,100 RBC		

TABLE IV—CONT'D
SIMILAR TYPES—TREATMENT NOT COMPLETED

CASE	SEX	AGE	DIAGNOSIS	PROBABLE DURATION	DAYS ON TREAT- MENT	CHANGES IN				WEIGHT CHANGES POUNDS	COMMENTS
						HEMOGLOBIN SAHLI (%)	BEFORE	AFTER	RBC IN MILLIONS		
11	F	37	Incomp septic abortion Pelvic peritonitis	8 da	30	34	35	35	2 80	3 35	Temp throughout colotomy and salpingectomy several wks prior to treat # ₁ Septic temp for 57 days # ₂
12	F	48	Bron pneumonia empyema & bleed hemorrhoids	2 mo	73	27	49	49	1 85	3 01	Died in uraemia No prog on Bl 107
13	M	16	Glomerular nephritis subacute attack	6 yr	67	38	38	38	1 93	2 08	Also received 20 gr quinine daily # ₂
14	F	29	Malaria	34 mo	32	26	66	66	1 13	2 95	Septic temp throughout Latio not determined Little prog
15	F	35	Pelvic peritonitis	1 yr	125	45	62	62	2 62	3 11	No improvement Latio to T B hosp
16	F	44	Bact endocarditis	1 yr	194	44	56	56	2 28	2 90	On treat during prog had severe postpart hemorrhage while on therapy # ₂
17	M	34	T B scapula Mult abscesses	4 mo	56	52	70	70	2 56	3 73	Still running septic temp * Septic temp no prog * Started on treat 18 days following delivery *
18	F	25	Anemia of pregnancy and post partum hemorrhage	8 mo	134	47	73	73	2 96	4 44	Temp for 2 wk while on treat no progress *
19	F	22	Incomp septic abortion	4 mo	40	30	59	59	2 14	3 52	Bedridden No prog although bl level man
20	M	16	Hodgkin's disease	10 yr	23	46	43	43	2 83	2 94	Recurrent low grade temp profuse and irregular menses # ₂
21	F	41	Purpural sepsis	15 da	64	27	67	67	1 58	3 40	
22	F	16	Unresolved puer	5 wk	28	61	64	64	3 07	3 25	
23	M	35	T B spine	1 yr	81	46	46	46	3 33	3 32	
24	F	30	Septic abortion	21 da	90	40	68	68	1 85	3 56	
Total					1047	593	816	816	35 24	46 25	
Average Patient					747	42 3	58 3	58 3	2 51	3 30	
Average Net Gain						160			0 79		
Average Daily Gain						0 22% Hb (Sahl)			10,700 RBC		

* Indicates female M Indicates male †400 cc blood transfusion 1 week prior to treatment # 1 Very little progress to whole liver and the alcohol soluble liver extract prior to present medication # 2 Cases that did not return for further observation or treatment *Cases still on treatment and observation

TABLE IV A

RESULTS OBTAINED IN CASES OF SECONDARY ANEMIAS OF THE "CHLOROTIC," IDIOPATHIC, TOXIC, AND INFECTIOUS TYPE (RESPONSES PRIMARILY ATTRIBUTED TO THE WHOLE LIVER IRON THERAPY)
TREATED WITH PREPARATION BL 105 1 OUNCE T I D

CASE	SEX	AGE	DIAGNOSIS	PROBABLE DURATION	DAYS ON TREATMENT	CHANGES IN				WEIGHT CHANGES POUNDS	COMMENTS
						Hb MOGLOBIN SAHLI (%) BEFORE	AFTER	RBC IN MILLIONS BEFORE	AFTER		
1	F	41	Chlorotic type of anemia frequent rectal bleeding	16 yr	104	49	85	3.06	4.16	130 145	Had chills # 1
2	F	27	Purpura hemorrhagica	4 yr	65	73	80	3.60	4.28		Bruised easily No hemorrhages while on treatment # 2
3	F	28	Pelvic peritonitis Thrombophlebitis following hysterectomy	5 mo	73	65	83	3.60	4.38	108 119	Febrile throughout treatment
4	F	19	Rheumatic heart disease	3 wk	65	43	85	3.38	4.59	109 115	Mod temp throughout treat # 3
5	M	37	Acute lead pois Quadruplegia	2 mo	45	53	73	3.27	4.57		End of treat transferred
6	F	39	Chlorotic anemia also frequent rectal bleeding	3 yr	62	43	84	3.66	4.24	114 112	Had chills # 4
7	M	67	Decompensated heart Idio pathic anemia	4 yr	527	28	80	2.01	4.21	162 173	Digitized Active at end of treatment
8	F	24	Syphilis Arspenamine react (Dermatitis & Hepatitis)	7 dn	84	25	78	2.16	3.83	98 101	Liver still enlarged*
9	F	44	Decompensated heart Idio pathic anemia	5 yr	54	13	59	1.28	4.04		Kept digitized Made good prog *
10	M	59	Decompensated heart	1 yr	27	36	60	2.16	3.12		Digitized*
11	M	23	Ulcerative colitis	2 yr	52	44	76	2.63	4.93	133 160	
Total						472	843	30.83	46.67		
Average Patient						12.8	76.6	2.80	1.24		
Average Net Gain						34.0		1.44			
Average Daily Gain						4.14% Hb (Sahl)		18,300 RBC			

F Indicates female M Indicates male # 1 No response to 1 fraction G extract in 30 days # 2 Highest count in 1 year of observation # 3 Made little progress on BL 101 in 67 days # 4 No response to Fraction G liver extract in 1 month therapy * Cases still on treatment and observation

Faber No response was obtained from the administration of the 70 per cent alcohol soluble liver extract during one month's treatment. The hemoglobin and erythrocyte count became normal in both patients when changed to the concentrated liver-iron preparation. Cases 7, 10 and 11, three patients with marked cardiac decompensation and severe secondary anemia of unexplained etiology, responded well to preparation B1 105. The occurrence of this disease during the later years of life, with the history of recurrent attacks of cardiac decompensation and the associated anemia which complicated this picture only after several years, led us to believe that it was probably due to a dietary deficiency based on the gastrointestinal disturbances associated with the frequent attacks of cardiac failure.

Table V included a number of patients with far-advanced inoperable carcinomas with severe secondary anemia who were treated with preparation B1 105. A moderate increase in hemoglobin and red blood cells was noted in Cases 1, 3, 7, and 8. Although little or no progress was observed in most instances, the original blood level at the beginning of treatment remained fairly stationary up to the time of exitus.

In addition to the beneficial hematologic response obtained in the largest majority of patients on treatment, other noteworthy effects of the liver-iron and hemoglobin preparation were observed. The appetite was improved. There was also a corresponding increase of strength, weight, and in feeling of well-being. It was surprising to observe these changes, occasionally, in several of the inoperable carcinomatous patients. The period of preoperative preparation in undernourished anemic patients with low-grade septic processes was definitely shortened, as well as the duration of convalescence from infectious diseases and operative procedures.

The summary of the reticulocyte response observed in the ninety-four cases in the "treated" groups treated with B1 105, showed that the most frequent rise was from 1.5 to 4.5 per cent, although in many instances a rise of from 8 to 10 per cent was noted. This excluded the reticulocyte average in the carcinomatous group with bone marrow metastasis, where a response as high as 18.6 per cent (Case 4) was observed, produced probably by the stimulation of the hematopoietic system by the metastasis.

Definite hematologic and clinical improvement as previously stated was observed in the largest majority of patients treated with the concentrated liver-iron and hemoglobin preparation. However, insuperable difficulties were encountered in evaluating the effectiveness of this preparation as a hemopoietic in the various types of secondary anemias treated. This can readily be seen when one considers the many complexities that entered into the pathogenesis of these anemias. This was further complicated during treatment because in many instances specific medical and surgical treatment was necessary. As yet no reliable biological tests have been discovered to evaluate the effect of such a preparation of liver-iron and hemoglobin other than its effect upon the regeneration of blood in experimental uncomplicated anemias.

Although we were impressed by the effectiveness of the concentrated liver-iron and hemoglobin preparation B1 105 in the largest majority of "treated" cases as compared to the "control" group receiving a known weak formula, and

SECONDARY TABLE V
RESULTS OBTAINED IN CASES OF SECONDARY ANEMIAS IN CASES OF MALIGNANCY TREATED WITH PREPARATION BL 105 1 OUNCE T I D

CASE	SEX	AGE	DIAGNOSIS	PROBABLE DURATION	DAYS ON TREATMENT	CHANGES IN						COMMENTS
						HEMOGLOBIN SAHLI (%)		RBC IN MILLIONS				
						BEFORE	AFTER	BEFORE	AFTER			
1	F	48	Inoperable Ca of cervix	1 yr	90	28	48	1.55	2.42	5 yr treat mod vag bleed *		
2	M	69	Ca of prostate Mult bone metas	6 mo	17	35	43	1.40	1.37	Patient died		
3	F	30	Metrlg Inoperable Ca of cervix	8 yr	86	27	60	3.15	3.36	2 yr treat		
4	M	57	Ca of stomach Mult bone metas	7 wk	48	21	16	05	7.2	Patient died		
5	M	76	Ca of stomach with bleed & liver metastasis	1 yr	78	30	27	2.40	1.96	†		
6	M	65	Ca of stomach	2 yr	35	43	47	2.70	2.53	Patient died		
7	M	63	Ca of stomach	2 yr	128	19	40	1.72	2.24	Patient died from gastric humor		
8	M	70	Ca of stomach	6 mo	28	24	55	1.13	2.93	*		
9	M	40	Mysarcoma of chest wall	6 yr	11	39	34	2.75	2.72	Removed tumor mass		
10	F	43	Mult myeloma	6½ mo	54	33	33	1.62	1.42	Patient died		
Total					575	299	393	19.43	21.97			
Average Patient					57.5	29.9	39.3	1.94	2.20			
Average Net Gain						9.4		26				
Average Daily Gain						10% Hb (Sahlb)		4,521 RBC				

* Cases still on treatment † Did not return for further observation 1 Indicates female M Indicates male

TABLE VI
BLOOD CHANGES OCCURRING IN PATIENTS WHO HAVE BEEN OFF OF TREATMENT FOR VARIOUS PERIODS OF TIME PREVIOUSLY TREATED WITH FERRIC ION, BL 105 AND DISCHARGED IMPROVED

CASE	SEX	AGE	REFERENCE TO PREVIOUS TREATMENT TABLE/CASE	INTER-TREATMENT (DAYS)	CHANGES IN			COMMENTS	DAYS ON TREATMENT	Hb %	RBC IN MILLIONS
					HEMOGLOBIN (SAHL) % BEFORE AFTER	RBC IN MILLIONS BEFORE AFTER	WEIGHT CHANGES POUNDS				
1	F	21	T 2 C 1	182	86 82	4.35 4.03	113 127	No complaints	—	81	—
2	F	40	T 2 C 3	49	83 74	4.53 4.06	118 130	No complaints	36	78	4.12
3	F	20	T 2 C 4	182	81 66	4.25 3.39	100 95	Pain in lower left quadrant Appetite poor	—	—	4.00
4	F	26	T 2 C 5	139	84 84	4.18 4.01	150 146	No complaints	—	71	3.67
5	F	38	T 2 C 6	139	81 64	4.13 3.74	199 203	No complaints	—	—	—
6	F	49	T 2 C 7	154	81 82	4.18 4.39	184 187	Atc liver 1 time 1 week	—	81	4.01
7	F	27	T 2 C 9	104	83 69	4.53 3.22	104 102	No complaints	14	Did not return	—
8	F	29	T 3 C 2	139	80 65	4.40 3.85	160 166	No complaints	Did not return	Did not return	—
9	F	38	T 3 C 3	149	80 78	4.24 3.81	162 170	No complaints	35	80	4.26
10	F	38	T 3 C 5	175	86 74	4.24 3.83	136 145	No complaints	—	84	4.52
11	F	43	T 3 C 8	182	85 70	4.11 3.93	135 137	No complaints	—	—	—
12	F	40	T 3 C 11	190	81 81	4.55 4.12	206 204	Eating 1 lb liver t 1 d	28	80	4.12
13	F	39	T 3 C 13	188	83 78	4.37 3.06	120 175	Complaints of weakness	—	80	—
14	F	32	T 3 C 15	111	84 72	4.71 3.38	110 121	No complaints	28	Did not return	—
15	F	27	T 3 C 16	78	83 84	4.91 4.10	161 165	No complaints	21	62	3.95
16	M	43	T 3 C 16	49	80 53	4.49 2.79	171 169	Rectal bleeding past 3 weeks	56	81	3.59
17	F	46	T 3 C 18	163	80 65	4.06 3.53	163 173	No complaints	—	—	—
18	F	31	T 3 C 20	64	86 89	4.30 4.26	99 105	Atc liver 3 to 4 times a week up to past 3 weeks	40	82	4.21
19	F	47	T 3 C 24	14	85 71	4.28 4.14	199 205	No complaints	49	84	4.05
20	M	52	T 4 C 1	203	81 75	4.33 2.82	170 182	No complaints	—	—	—
21	F	37	T 4 C 6	105	83 82	4.10 4.15	173 200	No complaints	—	—	—
22	F	18	T 4 C 7	49	83 74	4.53 4.06	127 134	No complaints	Did not return	Did not return	—
23	F	30	T 4 C 8	91	83 74	4.14 3.35	130 137	No complaints	26	80	4.05
24	F	40	T 4 C 9	77	86 78	4.89 3.81	176 176	Complaints of generalized pains and aches	40	83	4.10
25	F	41	T 4 A C 1	167	85 76	4.16 4.07	145 147	Received 1/2 oz Bl 105 daily during interval	25	80	4.55
26	F	28	T 4 A C 3	199	83 71	4.38 3.41	119 126	Pain in lower left quadrant Swelling of left leg	14	77	4.16
27	F	19	T 4 A C 4	221	85 68	4.59 3.12	115 111	No complaints	Did not return	Did not return	—
28	M	67	T 4 A C 7	105	80 60	4.21 3.02	173 180	No complaints on digitals	21	80	4.08

*The responses obtained in patients with recurrent anemias after treatment was again instituted are tabulated in the last three columns

the good response obtained in eleven cases of secondary anemias classified under Table IV-A, which was primarily attributed to the administration of preparation Bl 105, conclusive evidence as to its effectiveness in blood regeneration in many of the secondary anemia patients was still lacking.

For this reason it was considered important to determine the hematologic changes, if any, that occurred in patients when off of treatment at various intervals of time, previously "treated" and discharged with a normal hemoglobin and red blood cell count. Letters were sent to all of the patients, asking them to return to the clinic for a check-up. Of the twenty-eight patients who returned, the intervals off of treatment varied from fourteen to two hundred and twenty-one days. All types of secondary anemia previously classified in Tables II, III, IV, and IV-A were well represented in this group. Although noteworthy gains in weight were observed in most instances, twenty-one patients showed a definite drop in hemoglobin and red blood cells while off of treatment (Table V). All of these patients were in apparent good health except one (Case 16) who had frequent rectal bleeding during the prior three weeks which was probably responsible for the recurrent anemia. The hemoglobin and red blood cell count remained normal in only seven of the twenty-eight patients. Three of these ate liver two to three times a week after having been discharged from the clinic.

Treatment with the concentrated liver-liver and hemoglobin preparation Bl 105 was again instituted in the sixteen patients with recurrent anemias previously treated and discharged as normal. The increase in hemoglobin and erythrocytes (Table VI) obtained in fifteen of these sixteen patients after being treated again with preparation Bl 105 under home conditions, we feel is conclusive evidence of the effectiveness of this preparation in the regeneration of hemoglobin and red blood cells as there were no other factors to which their recovery could be attributed.

SUMMARY

One hundred and twelve patients with secondary anemia due to various causes have been treated with a preparation consisting of concentrated whole liver, iron in glycerol, and defibrinated blood. The rationale of the preparation used is discussed. A large percentage of this group of patients showed hematologic and clinical improvement on receiving the whole liver-iron preparation and other indicated therapy. We can only state with confidence that the improvement of eleven of the patients can be accounted for primarily on the basis of having received the whole-liver-iron preparation. Further evidence as to the effectiveness of this preparation was shown by the drop in hemoglobin and red cells when treatment was discontinued in twenty-one patients representing the various groups previously treated and discharged as normal. Hematologic recovery was obtained when treatment was again instituted under "home conditions." Our study has impressed us particularly in regard to the numerous and almost insurmountable difficulties inherent in the problem of determining clinically the value of a therapeutic agent in the hemorrhagic and idiopathic secondary anemias.

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LIVER EXTRACT IN THE TREATMENT OF DIABETES MELLITUS*

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- I THE EFFECT OF DRIED LIVER EXTRACT ON FIVE DIABETIC CHILDREN
 - II THE EFFECT OF DRIED LIVER EXTRACT ON ADULT DIABETIC PATIENTS
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INTRODUCTION

IN 1927 and 1929 Murphy and Blotnei^{1, 2} reported the effect of whole liver on the blood sugar level of diabetic patients. A year later³ they reported the effect of certain liver extracts on the blood sugar of such patients. This second paper contains an excellent review of the literature on the beneficial effect of liver extract in the treatment of diabetes, referring particularly to the work of Gilbert and Carnot,⁴ Jousset,⁵ Lamoreaux,⁶ Gilbert and Leieboulet⁷ and Lasance.⁸ Experiments had been carried on by the French investigators^{3, 4, 5, 6, 7, 8} using aqueous, alcoholic and saline extracts of liver in patients with diabetes mellitus. The conclusions were much alike. Apparently the oral administration of liver extracts had an appreciable influence on the glycosuria in a number of diabetic patients, the effect varying according to the case. During liver therapy the glycosuria disappeared or was diminished in some cases, whereas in others it was increased. The effects of the substance continued for a period of time after the treatment ceased. They felt that the cases which were favorably influenced by liver extract therapy were associated with a functional insufficiency of the liver while the cases which did not show improvement or had been made worse were those in whom the glycosuria depended on a hyperactivity of the liver. Lamoreaux⁶ suggested that liver either acted by increasing the accumulation of glycogen in the liver or that acting on the whole organism it resulted in a more rapid destruction of sugar.

Blotnei and Murphy³ treated four diabetic patients with liver extracts over varying periods of time. The determinations were made for twenty-nine days on one case, thirty-one days on the second, sixty-eight days on the third case and five months on the fourth case. In the patient receiving the liver extract for twenty-nine days, the blood sugar level was lower than previously, and when seven units of insulin were injected twice daily, the blood sugar level was slightly lower than during the period of liver extract therapy alone. In the second case, the blood sugar level during liver extract therapy was at a slightly higher level than during the period when thirteen units of insulin was taken. In the third case the blood sugar remained at a lower level when liver extract was taken and

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increased when it was omitted. In the fourth case the blood sugar was stabilized by the liver extract and the urinary sugar was decreased. In their summary they suggest that liver contains a blood sugar reducing substance active when taken by mouth.

In studying the effect of any drug other than insulin on a diabetic patient the observations should be carried on over a considerable period of time. There are well recognized reasons for this. A properly treated diabetic on diet alone will gradually regain a certain amount of his carbohydrate tolerance, and if he adheres strictly to diet he may remain sugar free with a relatively low blood sugar level for an indefinite length of time. In the same way certain diabetic patients treated with insulin and diet may omit insulin for varying periods of time, sometimes several months, and will remain sugar free, their tolerance having been greatly increased during the period of insulin therapy. Obviously one cannot attribute the fact that the urine remains sugar free to the drug one is endeavoring to substitute for insulin until the patient has been observed for a period of several months.

In addition to the direct effect of liver and liver extracts on the blood sugar level, there has been a considerable amount of experimental work on the effect of liver injury on carbohydrate metabolism. Ravdin⁹ found that the liver of jaundiced dogs kept on a mixed diet containing considerable amounts of carbohydrate contained only 1.4 per cent glycogen per 100 gm. of liver, whereas the liver of normal dogs on the same diet contained an average of 5.5 per cent glycogen. The blood sugar curves in these animals were distinctly elevated after the production of jaundice. He felt that his results indicated that the liver of the jaundiced animal was unable to store glycogen to the same extent as that of the normal dog. His work confirmed the finding of various other investigators, including Hetenyi,¹⁰ who showed that in patients with liver disease, the blood sugar rise after 100 gm. of dextrose was higher and more prolonged than in normal people. Ferguson¹¹ found the same true in dogs after experimental ligation of the common duct and cholecystectomy. Observations of this kind indicate that a deficiency of the glycogen forming function of the liver may result from injury or disease of that organ. It is possible that such a deficiency may occur before there is any clinical or laboratory evidence of liver dysfunction. Forsgreen,¹² in normal rabbits, found that the percentage of glycogen and bile varied inversely to each other, the bile being greatest in amount when the glycogen was least. The latter was first deposited around the central veins of the lobule and remained there longest. One might infer, from these observations, that the primary disturbance in glycogen storage follows some pathologic change in the region of the central veins.

Chloroform and ether anesthesia, which markedly injure the liver, give rise to a pronounced hyperglycemia. Rosenthal and Bourne¹³ found a definite interference with hepatic function after such anesthesia. Davis, Hall and Whipple^{14, 15} and also Ravdin⁹ found that liver cells regenerate rapidly on a high carbohydrate diet. This makes one realize again the importance of diet to the diabetic individual and suggests that the loss of glycogen from the liver in diabetic patients is in itself a cause of liver damage.

TABLE I
COMPARISON OF TETRAMETHYLENE AND NONFIRMEN TABLE BLOOD SUGARS IN PATIENTS TREATED WITH LIVER EXTRACT, INSULIN AND LIVER LACTATE,
DIET ALONE AND INSULIN

LIVER EXTRACT				INSULIN AND LIVER EXTRACT				DIET ALONE				INSULIN			
TOTAL BLOOD SUGAR MG	NONFIRMEN BLOOD SUGAR MG	FIRM BLOOD SUGAR MG		TOTAL BLOOD SUGAR MG	NONFIRMEN BLOOD SUGAR MG	FIRM BLOOD SUGAR MG		TOTAL BLOOD SUGAR MG	NONFIRMEN BLOOD SUGAR MG	FIRM BLOOD SUGAR MG		TOTAL BLOOD SUGAR MG	NONFIRMEN BLOOD SUGAR MG	FIRM BLOOD SUGAR MG	
107.1	16.6	90.5		97.9	13.1	84.7		80.0	13.8	66.2		52.3	11.1	41.2	
110.3	18.0	92.3		104.1	11.9	92.2		81.0	13.5	67.5		73.2	11.1	62.1	
120.0	12.0	108.0		136.3	13.6	120.7		83.0	14.3	68.7		83.3	20.8	62.5	
121.0	12.1	108.9		148.5	12.5	136.0		87.0	14.7	62.3		95.2	14.7	80.5	
125.0	11.1	113.9		156.2	14.7	141.5		90.0	18.9	71.1		105.0	15.6	89.4	
128.1	12.8	115.3		166.6	10.4	156.2		105.0	12.5	92.5		108.7	13.1	95.6	
128.1	13.1	115.0		176.4	11.9	164.5		114.0	13.8	100.2		116.2	10.6	105.6	
130.3	13.8	116.5		187.5	9.6	177.9		131.5	14.1	117.4		117.1	12.0	105.1	
133.9	11.0	122.9		194.8	11.1	183.7		137.0	12.5	124.5		129.0	15.6	113.4	
141.4	10.0	131.4		194.8	15.1	179.7		142.0	15.6	126.4		130.3	11.9	118.4	
141.4	12.5	128.9		202.7	10.8	191.9		142.0	14.2	138.1		137.1	10.6	124.5	
141.4	9.6	131.8		211.2	22.7	188.5		166.0	13.3	152.7		146.2	10.2	126.1	
145.7	12.1	129.3		211.2	15.6	195.6		166.0	15.4	150.6		137.6	11.8	123.8	
145.7	11.9	133.8		234.5	11.3	223.2		210.0	10.0	200.0		141.4	16.0	125.4	
150.0	12.5	137.5		250.0	12.5	237.5		250.0	12.0	238.0		150.0	10.0	132.5	
156.2	9.6	146.6		270.0	12.5	257.5		287.0	13.2	271.8		157.8	11.7	146.1	
163.0	12.6	150.4		263.1	12.1	251.0						166.7	15.1	151.6	
164.8	12.1	152.7		312.5	10.0	302.5						168.5	20.4	149.1	
166.6	11.3	155.3		312.5	13.8	298.7						169.0	27.7	141.3	
176.4	10.8	165.6										187.5	28.4	159.1	
200.0	13.2	186.8										200.0	14.7	183.3	
200.0	11.9	188.1										210.0	12.7	197.3	
												219.0	13.1	205.9	
												235.0	15.4	219.6	
												235.0	14.3	220.7	
												243.9	16.6	227.3	
												246.1	15.3	230.8	
												263.1	11.0	232.1	
												272.7	14.7	258.0	
												277.0	13.2	261.8	
												291.1	15.1	279.0	
												300.0	12.5	287.5	
												300.0	13.1	286.9	
												312.4	13.8	293.2	
												319.1	16.6	295.8	
												326.0	11.3	307.8	
												333.1	12.5	313.5	
												378.1	10.4	322.9	
												401.7	20.4	318.0	
												411.1	11.0	382.8	
												411.1	11.5	420.0	

PROCEDURE

The effect of the powdered liver extract has been observed in young and adult diabetic patients. The extract was made by the Lederle Laboratories using a slight modification of the procedure described by Blotner and Murphy.³ In our observations we used mainly the dried extract as the original observers found this as effective as the moist paste and it was much easier for the patient to take. The amounts of extract used in each case are reported in the equivalents of the amount of raw liver from which the extract was derived. Usually 400 gm of raw liver yielded about 25 gm of the dried extract. The patients observed received the equivalent of from 400 to 1600 gm of raw liver. In some cases the dose was increased to the equivalent of 2000 gm. The liver extract was given in divided doses one hour before meals. In some instances a dose was given on retiring at night. The patients had been treated in the diabetic clinics of the University and Bellevue Hospital Medical College, New York University, and the Third (New York University) Medical Division, Bellevue Hospital, for periods varying from two years to two months prior to treatment with liver. The young diabetics ranged in age from ten to nineteen years. The adult group included patients from thirty-four to sixty-one years of age. It has been suggested by several investigators that there are two types of diabetes, one in which the predominant disturbance is that of the oxidation of carbohydrate, the other in which the predominant disturbance is one of storage. It was felt that these two groups *might* present the two types of disturbance, provided one agrees that these two types exist and are sufficiently differentiated. The younger group, which consists invariably of the very severe diabetic, would be the type where the predominating disturbance was oxidative.

The relation of the fermentable and nonfermentable blood sugars in patients on insulin, on insulin and liver, on liver alone and on diet alone was also studied.

Liver function tests were done on all but one of the patients. These are reported in Table II.

Both fasting and after breakfast blood sugar determinations were made. The latter are indicated in the charts by a No. 2 at the level of the blood sugar reading. All blood sugar determinations were done by the Folin-Wu¹⁶ method. The cholesterol determinations were done by the modified Bloor¹⁷ technique.

RELATION OF FERMENTABLE TO NONFERMENTABLE SUGARS IN THE BLOOD IN PATIENTS
TREATED WITH DIET, INSULIN, INSULIN AND LIVER EXTRACT, AND LIVER
EXTRACT ALONE

Table I shows that there is no significant alteration in the relation of the fermentable to the nonfermentable sugars of the blood in patients treated with and without insulin, or with liver extracts. The highest nonfermentable blood sugars were found in the group treated with insulin alone but the average of the group was only 2 and 3 mg higher than the other three groups. Apparently liver extract did not alter the ratio of fermentable and nonfermentable blood sugars.

RESULTS OF THE LIVER FUNCTION TESTS

All of the usual liver function tests were performed. In addition, on Cases 5, 6, 8, and 9 the recently reported test of Harrop and Baron,¹⁸ in which bilirubin is injected intravenously, was used. The patients who showed some interference with liver function (Cases 5 and 8 with bromsulphalein and Cases 5, 6, and 8 with the injected bilirubin) were not patients who showed any definite improvement as a result of liver extract treatment. The liver function tests were done by Dr. Norman Joffe.

THE EFFECT OF LIVER EXTRACT ON YOUNG DIABETICS

Five young diabetics were treated with liver extract. The cases were

Case 1C, female, aged 15 yr, M S

2C, female, aged 15 yr, R C

3C, male, aged 15 yr, A B

4C, male, aged 10 yr, V K

5C, male, aged 15 yr, B S

The cases are not reported in detail because of lack of space. All blood sugars on the children were taken two hours after breakfast. Tables II, III, IV, V, and VI show the blood sugar levels.

TABLE II
LIVER FUNCTION TESTS

CASE	UROBILINOGEN URINE	BROMSULPHALEIN	INJECTED BILIRUBIN	VAN DEN BERGH			ICTERUS INDEX MG PER CENT
				DIRECT	DELAYED	INDIRECT	
1	Normal	Less than 5 per cent					2.19
2	Normal	Negative		-		+	0.4
3	Normal	5 per cent			+		0.87
4	Normal	Faint Trace					
5	Normal	Negative	4th hr 23.7%	-		+	0.5
6	Normal	Negative	4th hr 59%	-		+	0.3
8	Normal	20 per cent	3rd hr 60%	-		+	0.5
9	Normal	Negative	4th hr Neg	-		+	0.6
11	Normal	Negative		-		+	1.0
14	Normal	Negative		-		+	0.7
18	Normal	Negative		-		+	0.6

SUMMARY OF CASE 1—Admitted to the diabetic clinic in August, 1929, at which time the child weighed 69 pounds. She required from 60 to 75 units of insulin to remain sugar free during 1929, the diet during this year being carbohydrate, 115 gm, protein, 50 gm, fat, 102 gm. The weight increased to 85 pounds during this time. During the year 1930 the carbohydrate in her diet

TABLE III
CASE 1C—M S

BEFORE LIVER EXTRACT THERAPY		DURING LIVER EXTRACT AND INSULIN		INSULIN ALONE AFTER LIVER EXTRACT	
BLOOD SUGAR MG	CHOLESTEROL MG	BLOOD SUGAR MG	CHOLESTEROL MG	BLOOD SUGAR MG	CHOLESTEROL MG
153 0	385 0	429 4	242 4	217 4	229 8
200 0		400 0	267 0	357 1	229 8
322 5	230 1	375 0	236 1	416 6	
384 0		428 5	202 0		
		288 4	256 4		
		300 0	233 9		
		306 1	272 1		
		500 0	238 0		
		428 5	242 4		
		326 0	233 9		
		405 4			
		385 0	246 9		
		340 9	248 7		
		272 7	253 7		
		500 0	261 5		

was increased to 150 gm, the fat averaged from 90 to 115 gm. The insulin requirement during this year varied from 60 to 70 units. In August of this year, the patient was admitted to the hospital in diabetic coma. Up to September, 1930, the weight of this patient had increased to 99 pounds. On September 17, 1930, the patient was receiving 57 units of insulin on a diet of carbohydrates, 150 gm, proteins, 65 gm, fats, 115 gm and dried liver equivalent to 400 gm was started. The blood sugars during the period of liver therapy are reported in Table II. The insulin was reduced to 45 units on September 24 and liver extract was increased to an amount equivalent to 1200 gm. of raw liver. The patient continued on 45 units of insulin without any appreciable effect on the blood sugar level and continued to spill sugar in varying amounts in after-breakfast specimens. On September 17, the patient showed acetone. On September 19, patient was taken off liver owing to the presence of acetone and large amounts of sugar in the urine and the insulin was increased to 60 units. At the time of this report this patient required 70 units of insulin daily. The dried liver extract had no effect on this patient's blood sugar level or on the glycosuria.

CASE 2C—R C was admitted to the diabetic clinic March 26, 1929. Her weight was 90 pounds, blood sugar 268 mg, diet carbohydrates, 111 gm, proteins, 55 gm, fats, 105 gm, and insulin 60 units daily. During the year 1929, the child remained practically sugar free on this diet and required never less than 60 units a day. Her weight increased to 96 pounds. During 1930, the carbohydrate in the diet was raised to 136 gm. and patient was carried on from 40 to 60 units of insulin daily. On September 17, 1930, the patient was placed on the liver paste the equivalent of 400 gm of raw liver and insulin was reduced from 40 to 25 units. The patient remained sugar free on the reduced insulin until October 2 at which time the after-breakfast specimen of urine showed 3 per cent sugar. Liver was increased to the equivalent of 600 gm of the paste and the patient carried on 20 units of insulin, but the after-breakfast specimen con-

tinued to show sugar. There was no appreciable reduction in the blood sugar level during the treatment. The liver paste was increased on October 20 to the equivalent of 1000 gm. of raw liver. On November 3, dried liver extract equivalent to 1200 gm. was substituted for the wet liver extract. The patient's insulin at this time was 20 units a day. On November 15, the patient showed both sugar and acetone in the after-breakfast specimen. The insulin was raised to 30 units. As the patient continued to *spill sugar* and acetone after breakfast, the insulin was gradually raised to 70 units. Liver was discontinued on November 29 and on December 2 the patient was sugar free in the twenty-four hour specimen and the after-breakfast specimen. At the present time the amount of insulin has been reduced to 52 units and the patient is sugar free throughout the day. The blood sugar after liver had been discontinued showed a marked fall.

TABLE IV
Case 2c—R. C.

BEFORE LIVER EXTRACT THERAPY		DURING LIVER EXTRACT AND INSULIN		INSULIN ALONE AFTER LIVER EXTRACT	
BLOOD SUGAR MG	CHOLESTEROL MG	BLOOD SUGAR MG	CHOLESTEROL MG	BLOOD SUGAR MG	CHOLESTEROL MG
268.0		241.0	278.2	197.3	317.4
262.0		272.7	251.6	120.0	266.6
333.0		131.5	261.5	110.3	220.0
250.0	263.0	256.4	261.5	57.3	229.8
230.8	256.4	238.2	242.4	187.5	226.0
333.3	256.4	205.4	206.4	157.8	208.3
		230.8	261.5	133.9	226.0
		205.4	238.0	88.2	
		300.0	242.4		
		340.9	226.7		
		277.7	187.7		
		250.0	187.7		
		337.1	250.7		
		326.0	242.4		
		300.0	253.5		

CASE 3C—A. B., male. History of diabetes since 1922. Treated in this clinic since January, 1929, at which time the patient weighed 105 pounds, his blood sugar was 118 mg., and he was able to stay sugar free on 30 units of insulin daily and a diet of carbohydrates, 140 gm., proteins, 60 gm., fats, 120 gm. Blood sugars during 1929 went up on one occasion to 327 mg. but otherwise were never higher than 250 mg. Insulin requirement varied from 22 units to 65 units daily. Weight increased during this year to 114 pounds. During the year 1930, up to the time of liver therapy, the patient required from 65 to as little as 35 units to remain sugar free, depending on the amount of exercise. In September, 1930, the patient was taking 50 units of insulin and the carbohydrates in the diet had been raised to 185 gm. The patient was sugar free during the day but tended to show sugar after breakfast. On September 10, 1930, the patient was started on liver paste the equivalent of 1000 gm. of raw liver and 20 units of insulin were omitted, so that the patient was taking 30 units of insulin daily. The patient was sugar free for seven days except after breakfast. During this time, however, he lost four pounds. On the seventh day the twenty-four hour

specimen contained 5 gm of sugar. The insulin was raised to 42 units. The patient continued to spill sugar for one week, the insulin was decreased to 40 units, then increased to 45 units. The patient on October 8 showed a concentration of 10 per cent sugar in his urine after breakfast, at which time the insulin was 45 units. The insulin was raised to 50 units and patient became sugar free. The insulin was cut to 45 units and the patient again spilled sugar. On October 21, at which time the patient was receiving 55 units of insulin, the after-breakfast specimen showed a 6 per cent sugar and 2 plus acetone with a blood sugar of 500 mg. Liver was withdrawn insulin was raised to 70 units daily, and the patient became sugar free, but the blood sugar continued high. Liver was omitted until November 11 at which time the patient was placed on the dried liver extract, the equivalent of 800 gm. of raw liver. The amount of insulin at this time was 60 units. The after breakfast specimen on November 15 showed both sugar and acetone. The patient was continued on this liver and insulin and was sugar free from November 18 to November 29 at which time the after-breakfast specimen showed 5 per cent sugar and 3-plus acetone. Liver was discontinued December 2. To render this patient sugar free it was necessary to raise the insulin to 82 units. This boy is extremely cooperative and has adhered strictly to his diet ever since he has attended our clinic. The diet at home was checked by the visiting dietitian. Liver obviously had no effect on the blood sugar of this young diabetic.

TABLE V
CASE 3c—A B

BEFORE LIVER EXTRACT THERAPY		DURING LIVER EXTRACT AND INSULIN		INSULIN ALONE AFTER LIVER EXTRACT	
BLOOD SUGAR MG	CHOLESTEROL MG	BLOOD SUGAR MG	CHOLESTEROL MG	BLOOD SUGAR MG	CHOLESTEROL MG
118.0		500.0	173.0	After First Period of Liver Extract	
222.0		375.0	233.9		
167.0		258.0	215.2		
166.0		394.7	190.5		
200.0		150.0	224.9	428.6	177.7
251.0		375.0	185.0	376.9	196.0
327.0		163.0	202.0	468.6	199.0
371.0	297.0	357.1	233.9	After the Second Period	
250.0	304.0	428.0	210.3		
285.0		157.8	177.7		
62.0	154.0	500.0	238.0		
156.0		Second Period of Liver		348.8	218.6
500.0				300.0	191.0
				400.0	173.0
				378.3	
		441.0	222.2	300.0	193.0
		365.0	207.0	250.0	166.6
		428.0	222.2	307.0	
		384.6	229.8	71.0	
				208.3	

CASE 4C—V K, aged ten, weight 79 pounds. Admitted to this clinic July, 1930. The patient was carried on a diet of carbohydrate, 140 gm, protein, 56 gm and fat, 89 gm. Insulin requirement varied from 20 to 25 units. On September 10, the patient was placed on the equivalent of 400 gm of dry liver

and the insulin was reduced to one dose of 10 units before breakfast. On September 13, the patient showed sugar in his after-breakfast specimen. The decreased dose of insulin was continued and the liver increased to the equivalent of 1200 gm. The patient was sugar free on September 20 but from then on showed sugar in his after-breakfast specimen. The total amount of insulin was increased to 16 units, then to 18, then to 20, then to 25. Liver was discontinued October 21. The patient required from 30 to 42 units of insulin to remain sugar free. The blood sugar level and glycosuria were not affected by the use of liver. At the time of this report this patient required 27 units of insulin daily and the carbohydrate in his diet had been raised to 165 gm.

TABLE VI
CASE 1C—V K

BEFORE LIVER EXTRACT THERAPY		DURING LIVER EXTRACT AND INSULIN		INSULIN ALONE AFTER LIVER EXTRACT	
BLOOD SUGAR MG	CHOLESTEROL MG	BLOOD SUGAR MG	CHOLESTEROL MG	BLOOD SUGAR MG	CHOLESTEROL MG
103.0	205.6	187.5	211.6	326.0	173.1
390.0		352.3	196.7	187.0	212.9
122.0		250.0	215.0	93.8	185.1
		272.7	211.6	172.4	211.6
		306.1	215.0	100.0	242.4
		182.9	222.2	91.7	222.7
		348.8	193.2	200.0	
		357.1	242.4	263.1	192.6
		441.0	193.2	220.5	168.7
		83.3	208.5	168.5	226.0
		327.8	187.7	394.7	
		319.1	192.4	159.6	277.7
				125.0	
				300.0	

CASE 5C—B S, aged sixteen. History of diabetes since 1926.

Treated in the diabetic clinic since 1929. The patient required an average of 65 units of insulin daily to remain sugar free on a diet of carbohydrates, 180 gm., proteins, 80 gm., and fats, 100 gm. The patient was hospitalized and the dried liver extract was started October 27, 1930, the equivalent of 1200 gm of the raw liver. The patient required varying amounts of insulin during this period, the lowest being 59 units daily. On this amount, the patient was sugar free but the fasting blood sugar level was extremely high, never being less than 400 mg. This high blood sugar level was also noted in Case 3C. The patient remained sugar free, the amount of liver in the form of the dried extract being raised to the equivalent of 2000 gm. Insulin was cut to 69 units and on November 12, the patient showed a faint trace of sugar. On November 14, 25 gm of sugar were spilled in the twenty-four hour period. The patient spilled again on November 17 and on November 20 and continued to spill from November 20 to December 5. The insulin during this time was raised to 75 units.

The patient was taken off liver on December 10 and was kept sugar free on 65 units of insulin daily. The blood sugar level remained high until December

29, at which time it was 75 mg. The blood sugar on January 2 was 83 mg. Insulin was then reduced to 50 units daily, then to 45 units and the patient was discharged from the hospital on 30 units of insulin daily. The present insulin requirement varies from 40 to 60 units daily.

TABLE VII
Case 5c—B S

BEFORE LIVER EXTRACT THERAPY		DURING LIVER EXTRACT AND INSULIN		INSULIN ALONE AFTER LIVER EXTRACT	
BLOOD SUGAR MG	CHOLESTEROL MG	BLOOD SUGAR MG	CHOLESTEROL MG	BLOOD SUGAR MG	CHOLESTEROL MG
300.0	242.4	267.8	242.4	535.7	196.3
272.7	272.1	500.0	266.6	428.6	215.7
		576.8	229.8		
		416.6	261.5		
		2nd Time on Liver			
		454.5	242.4	454.5	202.6
		454.6	203.9	428.5	193.2
		500.0	202.0	483.9	218.6
		468.7	225.9	454.5	192.6
		500.0	228.3	75.0	148.1
		500.0	261.5	83.3	
		535.7	271.0	52.3	222.2
		576.9	222.2	205.4	
		500.0	208.3	333.3	202.0
		384.6	186.7	164.8	182.6
		600.0	225.9	174.4	199.0
		468.7	258.3	87.2	177.7
		535.7	264.5	53.6	191.5
		576.9	224.0	441.0	199.9
		375.0		53.6	
		500.0	205.7		
		441.0	180.1		

SUMMARY OF THE CASES OF THE YOUNG DIABETICS TREATED WITH LIVER

Any one who has treated diabetic children realizes that the insulin requirement varies according to the child's activity and to whether or not any minor infection occurs. Four of these children were treated in the clinic and the insulin requirement was not materially altered during the period of liver therapy, nor was there any fall of the blood sugar level. On the contrary, three cases showed a definite tendency to a higher blood sugar level while on liver and insulin than when on insulin alone. One case was treated in the hospital. During the period of liver therapy no material change was effected in the amount of insulin, in fact less insulin was required to keep the patient sugar free after liver therapy was discontinued. The hospitalized case was one of the cases showing an elevated blood sugar level with no glycosuria during the period of liver therapy. In the study of these five cases, it seems justifiable to conclude that neither the dried nor the moist liver extract can replace or reduce the amount of insulin required by the young diabetic.

TABLE VIII
SUMMARY OF ADULT DIABETIC PATIENTS

CASE		AGE	SEX	ONSET OF DIABETES	PREVIOUS DIFF				PRESENT DIFF				TOTAL PERIOD ON LIVER (WAS)	DRIED LIVER AMT GIVEN IN EQUIVALENT OF GM OF RAW LIVER
					INS	C	I	F	INS	C	I	F		
1	L II	47	F	1920	12	135	56	102	5	117	60	87	41	1800
2	T W	51	F	1926	35	150	64	150	15	199	63	98	59	400 1200 1800
3	II B	42	F	1929	8	130	56	102	5	152	60	97	45	800 1600
4	A G	44	M	1925	8	142	78	116	5	112	78	111	15	800 1200
5	M S	61	M	1930	5	170	56	115	0	161	58	65	50	500
6	R C	46	M	1929	30	130	64	100	Not Known				23	1600
7	C B	34	F	1929	20	180	75	122	10	171	61	81	51	1200
8	R K	57	F	1930	15	110	56	90	10	110	56	90	40	800 2000
9	N N	39	M	1929	70	130	65	100	15	175	64	85	19	1600 2400
11	II G	55	F	1925	20	140	55	91	20	140	55	91	36	800 2000 1800
14	J B	52	F	1930	0	150	58	102	0	170	66	101	39	1200 1600
15	G N	53	F	1927	0	80	55	97	0	100	55	80	29	800
18	A W	35	F	1930	10	140	53	91	0	110	53	91	25	1200

THE EFFECT OF THE DRIED LIVER EXTRACT OVER A PROLONGED PERIOD OF TIME ON ADULT DIABETIC PATIENTS

The effect of the dried liver extract was observed on thirteen adult diabetic patients. The summary of these cases is shown in Table VIII. In several patients the moist and liquid extracts were used for short periods of time but were discontinued, as they were extremely unpleasant to take and as Blotner and Murphy's results did not show any difference in their effect from that of the dried extract. Cases 1, 6, and 9 were hospitalized for a period of time during the administration of the extract. Blood sugar, cholesterol, and urinary sugar determinations were made on all patients. The patients examined their urine by Benedict's qualitative method daily, and a twenty-four hour specimen of urine was examined each time the patient came to the clinic. In some cases, the patient returned weekly for examination, in others twice a week. The diet in each case was carefully checked. All of these patients weighed their food on scales and were instructed carefully about the necessity for accuracy and adhering to the diet as ordered.

The blood cholesterol determinations, done each time the patients reported for blood sugars, are not reported in detail owing to lack of space and also because there was no significant alteration as a result of liver therapy. The average blood cholesterol before and during liver therapy are reported in Table IX.

TABLE IX

AVERAGE OF THE BLOOD CHOLESTEROL DETERMINATION BEFORE AND DURING LIVER EXTRACT THERAPY ON THE ADULT PATIENTS

CASE	AVERAGE BLOOD CHOLESTEROL MG	
	BEFORE LIVER EXTRACT TREATMENT	DURING LIVER EXTRACT TREATMENT
1	251.8	267.9
2	226.0	210.0
3	254.7	268.0
4	290.2	246.3
5	211.1	188.4
6	303.0	281.2
7	237.2	227.1
8	244.4	249.2
9	254.2	261.1
11	233.3	255.4
14	276.0	244.4
15	232.3	215.9
18	221.6	207.5

Of the 13 cases investigated all but 2 (Cases 14, 15) were on insulin prior to receiving liver extract. These 2 patients were on liver for a period of thirty-nine and twenty-nine weeks respectively, Case 14 receiving the equivalent of 1200 to 1600 gm of raw liver and Case 15 receiving the equivalent of 800 gm of raw liver. The charts on these patients show that there was no effect on the blood sugar level during the period of liver therapy (Charts 14 and 15).

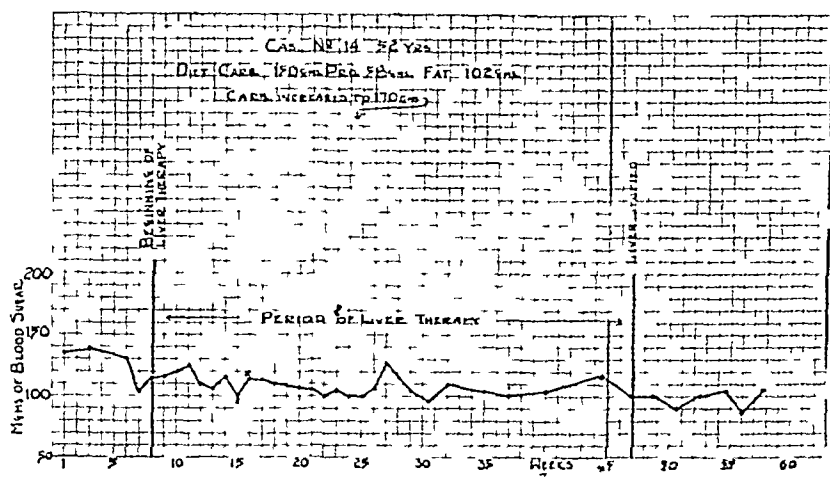


Chart 1

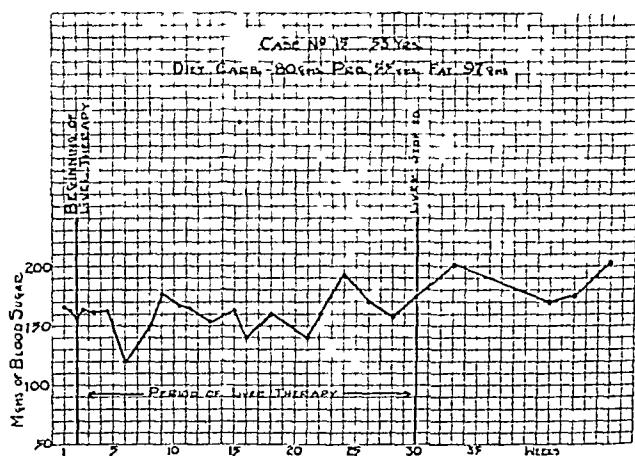
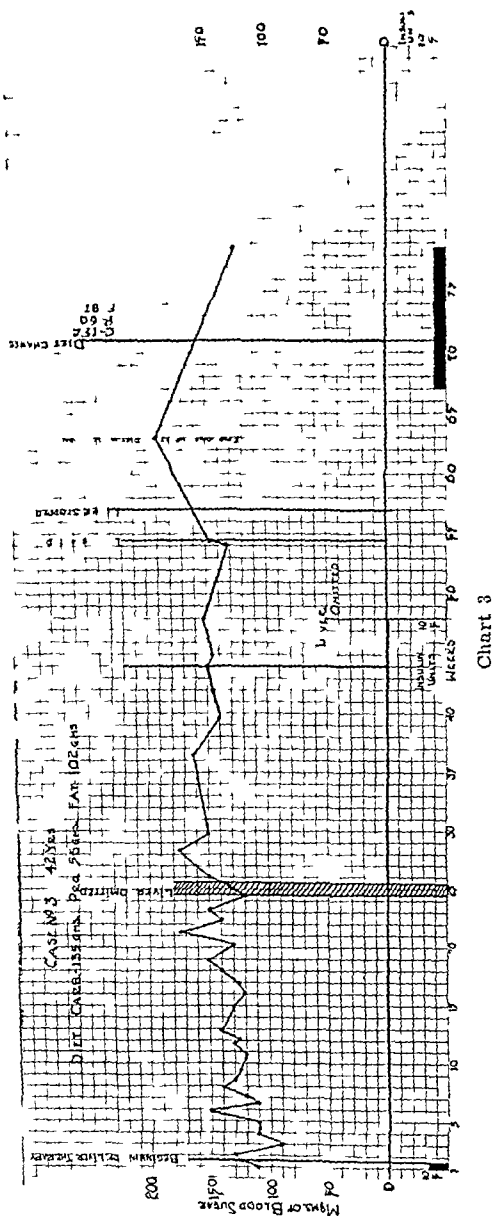


Chart 2

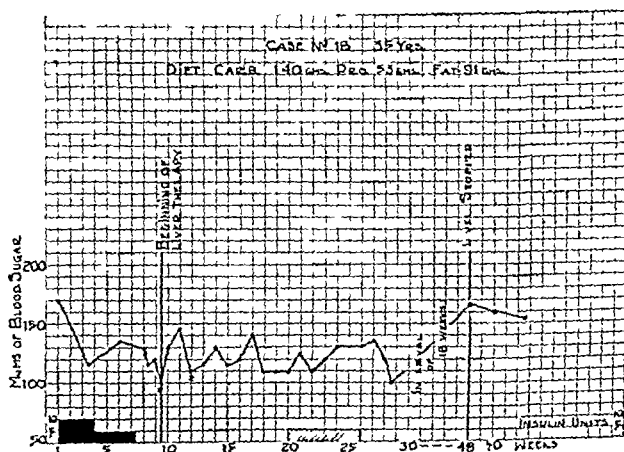
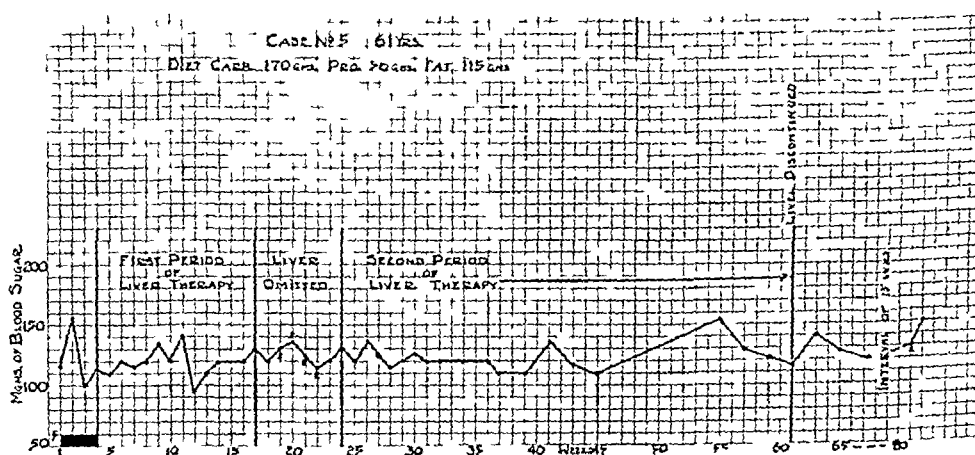
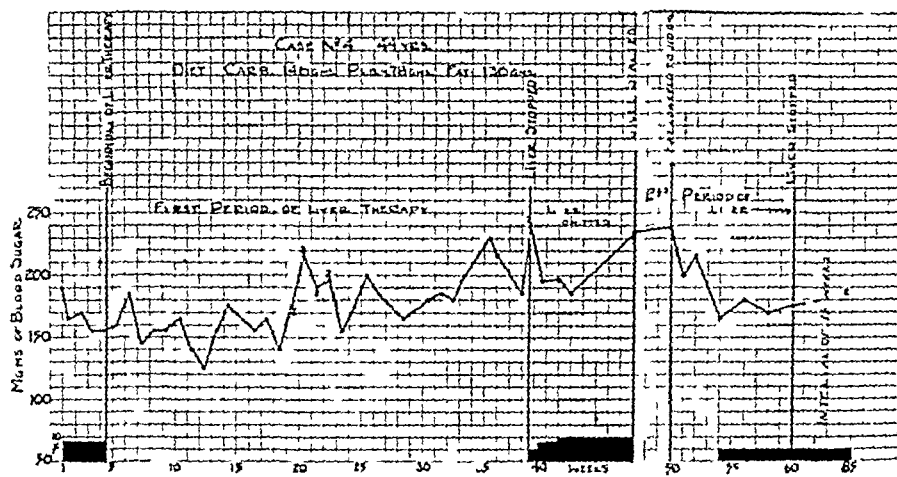
Four patients (Cases 3, 4, 5, and 18) had required ten units of insulin or less daily. Following the use of liver extract, for periods of not less than twenty-five weeks and extending in 3 cases for forty-five weeks or more, there was no significant effect on the blood sugar level. It is true that all 4 patients were able to omit insulin for a period of time but in the treatment of diabetic patients who are on small doses of insulin this is a circumstance that often occurs and if the diet is adhered to strictly, insulin may be needed for only short periods of time. This is further brought out in all of these cases by the fact that when liver was

discontinued and the patient was treated without either insulin or liver, there was no striking increase in the blood sugar level (Charts 3, 4, 5, and 18)

The remaining 7 patients (Charts 1, 2, 6, 7, 8, 9, and 11) required 12 or more units of insulin daily and in this group, Cases 2 and 9 showed results which

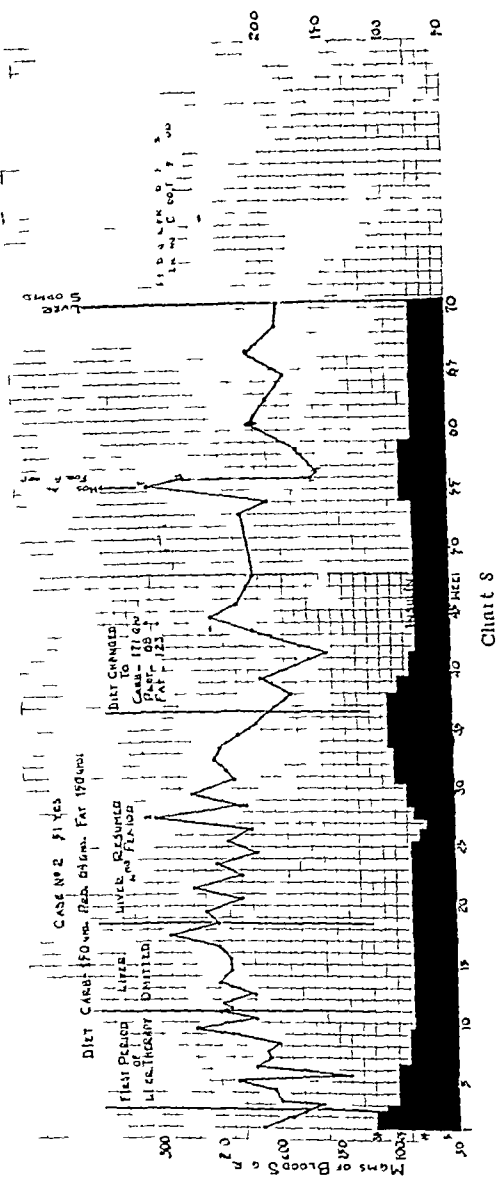
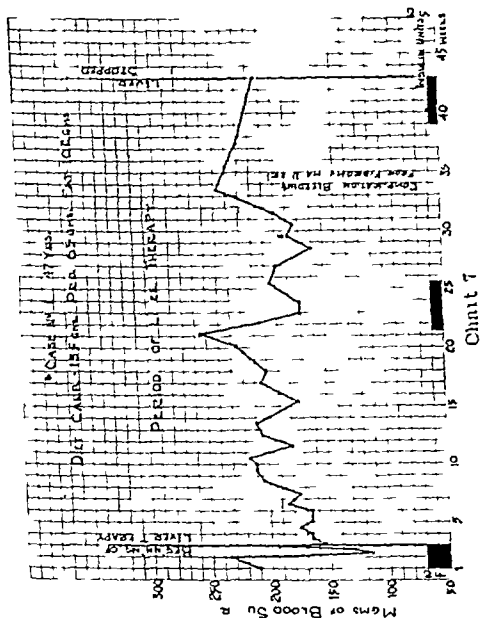


might be attributed to the effect of the liver extract treatment. In the other 5 cases the reduction of the daily insulin dose was not greater than what might be expected as a result of continued adherence to diet. In addition, these patients continued with blood sugars of the same level following withdrawal of liver



Case 2 was able to reduce insulin dosage from 35 to 15 units daily. After this patient had been treated for a period of thirty five weeks, the fat in the diet was reduced from 150 to 123 gm and the carbohydrate increased from 150 to 171 gm. Following this change in diet there was a greater fall in the blood sugar level than had been encountered during any period of the treatment. This patient is at present tolerating a diet of

Carbohydrate	200 grams
Protein	65 grams
Fat	100 grams



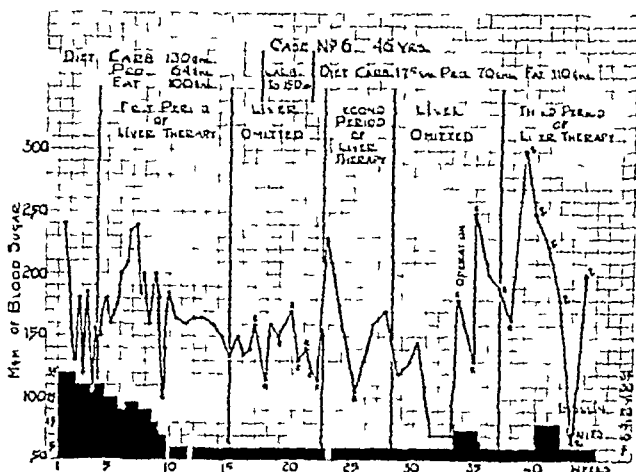


Chart 9

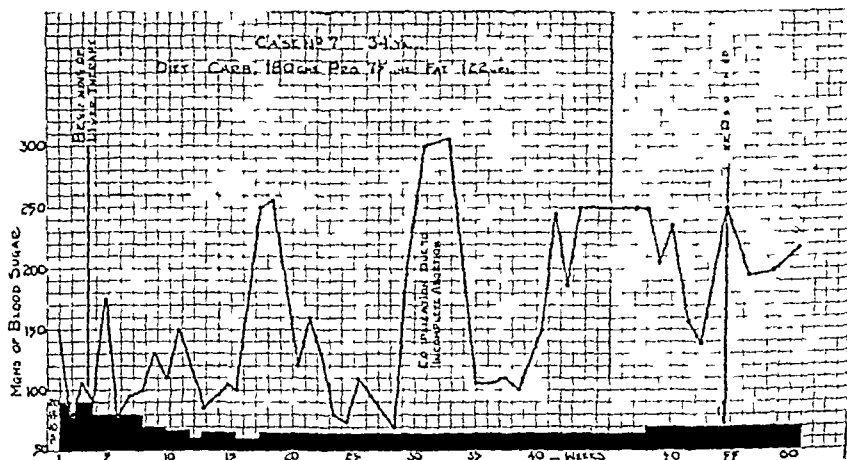


Chart 10

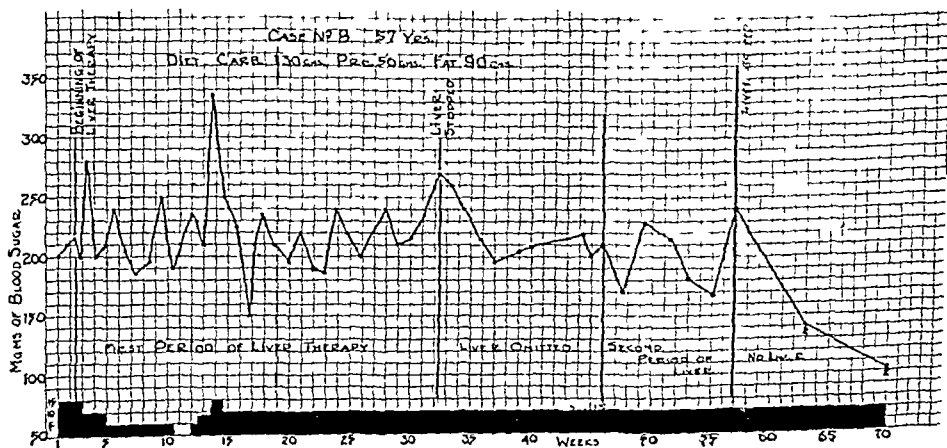


Chart 11

and has remained sugar free on a daily dosage of 15 units of insulin. Case 9 is a patient who had been treated in our clinic since 1929 and had required an average of 70 units of insulin daily. During his first period of liver therapy, it was possible to reduce the insulin to 25 units daily. When liver extract was omitted, the blood sugar rose, and it was again necessary to increase the amount of insulin. During this period the patient was kept sugar free on 55 units of insulin, below which amount he invariably spilled sugar. During his second pe-

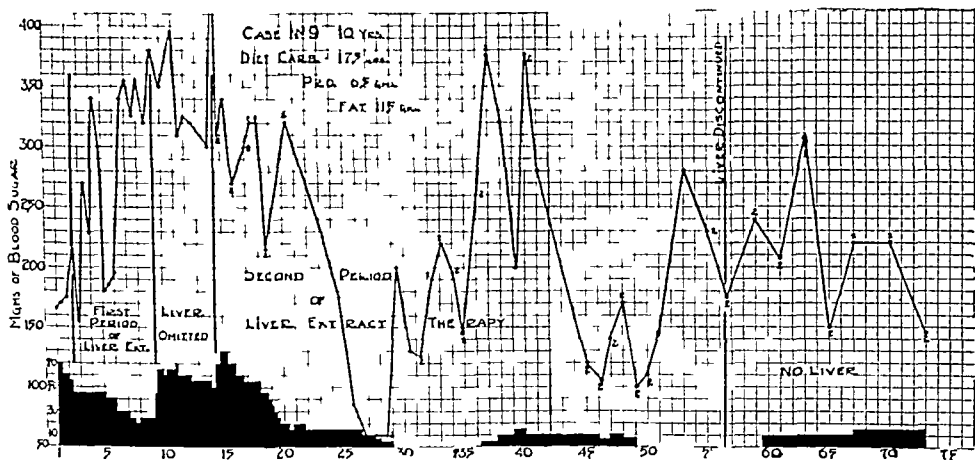


Chart 12

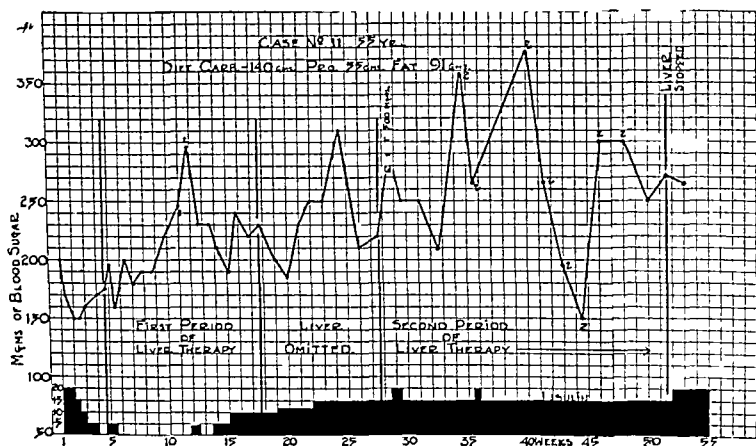


Chart 13

riod of liver therapy it was possible to reduce the insulin to 5 units daily and the blood sugar at this time was lower than at any time during the two years that the patient has been under observation. When insulin was withdrawn, the blood sugar immediately rose. Liver has been discontinued in this patient, and he has been on insulin alone for a period of over five months. His blood sugar level has remained below 200 mg and he has been kept sugar free on a dose of 15 units daily.

SUMMARY AND DISCUSSION

Our results do not substantiate the findings of Blotner and Murphy^{1, 2, 3}. In the young patients observed neither the dry nor the moist liver extract had any effect on the hyperglycemia, nor was it possible to reduce the insulin requirement during the period of liver therapy.

Of the adult patients only Case 9 seemed to show any marked improvement while receiving liver extract. In Case 2 it was possible to reduce the insulin but the hyperglycemia was affected more by the change in diet than it was by the liver therapy.

While these observations have been in progress other investigators have reported a similar lack of effect of liver extract in diabetes. Bowen and Sly¹⁹ investigated the effect of whole liver and liver extract on the respiratory quotient of two diabetic patients and found no evidence that either had a favorable influence on the diabetes.

DePencier, Soskin and Best²⁰ studied the blood sugar and sugar excretion of depancreatized dogs after whole liver and were unable to observe any favorable results. Certain of the German investigators Habs,²¹ Beitram, Horwitz and Wahnau²² reported that neither liver nor liver extract have any appreciable influence on the blood sugar or glycosuria in diabetic patients.

In the treatment of diabetes one is always impressed with the ability of the patient to increase his carbohydrate tolerance by adherence to diet and insulin, and if these patients are followed over a period of time the results are, in the majority of cases, striking. This makes it difficult to draw any conclusions as to the effect of a given type of therapy on the course of the disease. In this instance the patients were observed for periods of not less than one year and received the extract for never less than five months and in most of the cases for more than five months. Therefore, it seems reasonable to conclude that if any improvement were to occur it would have taken place during this prolonged period of observation. In view of this fact one does not feel justified in attributing to liver extract any effect similar to that of insulin on the hyperglycemia or glycosuria of diabetic patients.

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THE EFFECT OF LIVER EXTRACT ON BILE PIGMENT FORMATION*

M S KIM, M B, M S, CHICAGO, ILL

THERE are a number of reports in the literature suggesting that liver extract may have some effect on bile pigment metabolism. Why an apparent retention of bilirubin occurs in pernicious anemia, as is demonstrated by the icteric index and lemon yellow tinting of the tissues, is not known. That it is a definite feature of the disease is shown by the fact that when liver extract is given to the patient suffering from pernicious anemia, the icteric index decreases along with the reticulocyte response and increase in erythrocyte count. Murphy, Monroe and Fitz,¹ who first recorded this fact, suggested that the disappearance of bilirubin from the blood and tissues might mean that it was being utilized in the formation of hemoglobin by the bone marrow in response to the liver treatment. The other possibilities are that the liver extract might increase the elimination of bile pigment in the bile and that it may prevent excessive formation of bile pigment. Verzar² has suggested that bilirubin is the "hematopoietic hormone" and being a by-product of red cell destruction, regulates their formation and liberation into the blood stream. He reports that in rabbits, bilirubin in small doses stimulates erythropoiesis and in large doses inhibits, and that in anemic rabbits, regeneration of erythrocytes occurs more rapidly if bilirubin is administered. It remains to be proved whether this concept is true, however, it is related theoretically to the apparent retention of bilirubin in pernicious anemia. Jungmann³ and Schulten⁴ hold that liver therapy inhibits the hemolytic apparatus, since all signs of increased blood destruction are diminished. On the other hand, Singer⁵ has shown that the daily stercobilin output in the normal

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individual is not decreased on liver therapy, and Hariop and Barrion⁸ have found that the ability of the liver of the pernicious anemia patient to eliminate bilirubin injected intravenously is less than normal. Further, it is known that jaundiced and biliary fistula dogs frequently develop an anemia, and Whipple and Hooper⁷ have reported the biliary fistula dogs improve nutritionally when fed cooked liver. The constituent of liver that is responsible for this improvement is not known. It occurred to us, therefore, that the effect of liver extract on bile pigment output should be studied and that it should be ascertained if liver extract contained the principle responsible for the improvement of biliary fistula dogs.

METHODS

For this work biliary fistula dogs were prepared according to the method of Rous and McMaster.⁸ The dogs were kept in cages and given no exercise because of the known influence of exercise on bile pigment formation.⁹ The dogs were fed a stock diet of cooked yellow corn meal and bread mixed with bone broth. We used such a carbohydrate diet because the bile pigment elimination on such a diet is low¹⁰ and we thought significant changes would be more readily detected. The dogs were kept on the diet for one week before the operation and thereafter. During the test period the dogs were given the extract of 200 gm of liver (Eli Lilly, No 343) daily. The bile was collected from the balloon twice daily and the bile pigment estimated immediately. Thannhausen and Andersen's modification of the Van den Bergh method for serum bilirubin¹¹ was used. This method was compared with that of Rous and McMaster¹² and the results checked. The liver extract was found to contain no bile pigment and only a trace of bile salts.

RESULTS

Thirteen dogs were prepared with a biliary fistula. Eight lived from twenty-two to forty days, the other two died at twenty days from peritonitis due to a perforated duodenal ulcer. Three dogs were fed daily with "gastric mucin" (Aimour) and lived indefinitely in excellent condition.

All but three of the animals were in good condition during the period that data were collected. From one week to ten days after the operation, the bile and pigment output became uniform.¹⁰

Typical results are shown in Tables I and II. These were obtained on Dogs 5, 8, 10, and 11. A fairly uniform excretion of bile pigment occurred during the control and liver extract feeding periods, but occasionally an unexplainable increase in the twenty-four hour output of bile occurred.

The twenty-four hour output of bile on the diet fed varied from 5 to 15 cc per kilo body weight. The range of bile pigment output varied from 1 to 3 mg per kilo body weight in twenty-four hours.

The liver extract administration did not influence bile or bile pigment output in these dogs. Neither could it be said that the liver extract improved the nutritional condition of the dogs, but Fogelson's "gastric mucin" did. Duodenal or gastric ulcer has not appeared in another series of seventeen biliary fistula dogs on gastric mucin.¹³

TABLE I
SHOWING NO EFFECT OF LIVER EXTRACT ON BILE PIGMENT OUTPUT IN DOGS 5 AND 8

OPERATION DAYS AFTER	AMT CC BILE IN 24 HR.		AMT IN MG BILE PIGMENT IN 24 HR.	
	TOTAL	PER KILO BODY WT	TOTAL	PER KILO BODY WT
Dog 5—Male				
Stock diet				
8	150	9.7	22.5	1.5
9	140	9.0	25.6	1.7
10	155	10.3	21.9	1.5
Average	148	9.7	23.3	1.6
Stock diet with liver extract				
11	170	11.3	27.1	1.8
12	170	11.3	11.7	2.3
13	195	13.4	11.8	2.9
14	185	12.7	37.5	2.6
15	150	10.0	27.7	1.9
16	189	14.0	31.7	2.3
17	175	12.5	26.3	1.9
Average	178	12.2	33.0	2.3
Stock diet				
18	200	14.7	37.5	2.8
19	185	13.6	29.6	2.2
20	88	6.7	4.8	0.3
21	235	20.5	30.9	2.3
22	226	18.9	25.3	1.9
23	220	17.7	36.2	2.8
24	210	16.7	32.3	2.6
Average	195	13.8	28.1	2.1
Dog 8—Male				
Stock diet with liver extract				
8	125	8.2	22.9	1.5
10	125	9.0	26.3	1.8
12	140	10.0	40.4	2.9
14	100	7.5	34.9	2.6
Average	123.2	8.7	31.1	2.2
Stock diet				
9	140	9.6	27.6	1.9
11	140	9.8	43.6	3.0
12	130	9.4	33.6	2.4
15	98	7.5	34.2	2.6
Average	127	9.1	35.0	2.5

DISCUSSION

The data obtained show that liver extract does not influence the formation of bile pigment or bile output in biliary fistula animals. However, from this data it does not necessarily follow that liver extract might not have a favorable action on bile pigment elimination and metabolism in patients with pernicious anemia. This is substantiated by the work of Singer,⁵ who found that liver therapy did not influence the stercobilin output in the feces of normal subjects, which checks with our results, and by the recent observations of Paschkis and Diamant,¹⁵ who found a decrease in stercobilin output in pernicious anemia patients on the institution of liver therapy, the decrease in stercobilin output preceding or accompanying the improvement in the blood picture. Whether this decrease in bile pigment output in pernicious anemia patients is due to decreased blood destruction, or to the utilization of bilirubin in hemoglobin manufacture, or to some qualitative change in erythropoiesis is yet to be determined.

TABLE II

SHOWING NO EFFECT OF LIVER EXTRACT ON BILE PIGMENT OUTPUT IN DOGS 10 AND 11

OPERATION DAYS AFTER	AMT CC BILE IN 24 HR		AMT IN MG BILE PIGMENT IN 24 HR	
	TOTAL	PER KILO BODY WT	TOTAL	PER KILO BODY WT
Dog 10—Male				
Stock diet with gastric mucin, 30 gm per day				
10	85	11.6	21.2	2.9
11	80	10.0	18.7	2.4
12	105	13.4	18.7	2.3
13	95	12.3	17.8	2.3
14	90	11.7	19.2	2.4
Average	91	11.8	19.1	2.5
Stock diet with gastric mucin and liver extract				
15	100	13.1	17.8	2.3
16	90	11.7	19.5	2.5
17	100	13.1	22.0	2.9
18	95	13.5	16.1	2.3
19	105	14.6	19.2	2.6
Average	98	13.2	18.9	2.5
Dog 11—Female				
Stock diet with gastric mucin				
17	87	9.5	19.7	2.1
18	95	11.3	21.5	2.5
19	90	10.0	19.8	2.2
20	90	10.2	21.0	2.3
Average	90.5	10.3	20.5	2.5
Stock diet with gastric mucin and liver extract				
21	95	11.0	26.1	3.0
22	102	11.5	23.9	2.9
23	120	13.8	18.2	2.0
24	125	14.7	17.3	2.0
Average	110.5	12.7	21.4	2.5
Stock diet with gastric mucin				
27	135	15.3	19.3	2.1
26	120	13.8	21.8	2.6
27	135	15.3	19.3	2.1
28	135	15.5	24.9	2.8
Average	129	14.7	21.1	2.4

The range of bile excreted in twenty-four hours in our dogs was approximately the same as that found by McMaster and Rous,¹⁰ who observed a lower range of from 1 to 7 cc and an upper range of from 8 to 14 cc per kilo. Our results on bile pigment output are lower than those of Hooper and Whipple⁷ (9 mg per kilo) and those of Rous and McMaster^{8, 10} (6 to 7 mg per kilo). Our low figure is due to the diet, since the figures of the workers just referred to were obtained on diets containing meat and liver.

Duodenal ulcers were found in three dogs in this series of ten without mucin, perforation of the ulcer being the cause of death in two.¹³ The occurrence of duodenal ulcers in biliary fistula dogs has been reported by most workers and more recently by Berg and Jobling.¹⁴ How gastric mucin operates to prevent ulcer formation is not known.¹³

CONCLUSION

Liver extract (the fraction active in pernicious anemia) given by mouth does not influence the output of bile or bile pigment in biliary fistula dogs. Neither does it contain the active constituent of liver which improves the nu-

nutritional condition in biliary fistula dogs "Gastric mucin" maintains biliary fistula dogs in excellent nutritional condition

The author expresses his hearty thanks to Professor A. C. Ivy who suggested the problem and gave valuable advice, and is indebted to Dr. L. L. Walsh who assisted in the biliary fistula operations and to Dr. J. Sicks who gave advice concerning the methods of pigment estimation

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CARBON DIOXIDE CHANGES IN ALVEOLAR AIR AND BLOOD PLASMA OR SERUM AFTER SUBCUTANEOUS HISTAMINE INJECTION IN HUMAN BEINGS*

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HISTORY

IN THE work reviewed two main types of gastric stimulants have been used, food and histamine. Where the former has been used the CO_2 changes have usually been determined upon alveolar air. A rise in the CO_2 content of this air has been demonstrated by Higgins,¹ Dodds,² Bennett and Dodds,³ Brunton and Israels,⁴ Van Slyke, Stillman and Cullen,⁵ and Kauders and Poiges.⁶ The last two men say that this rise was found only in patients secreting an acid gastric juice.

Dodds and McIntosh⁷ were unable to find an increase in plasma CO_2 in food-stimulated cases but do report an increase in the CO_2 of the red blood corpuscles. Hubbard,⁸ in 1928, tested the effect of a carbohydrate high meal on patients who were suffering from "carbohydrate abnormalities" and found an increase in titratable alkalinity of the blood after such meals. Van Slyke, Stillman and Cullen⁵ found an increased plasma CO_2 in most of their cases after the ingestion of food.

The results following the use of histamine as a gastric stimulant seem to fall into two classes, according to the size of the dose. Where a relatively large amount was given, there was always a fall in plasma CO_2 , reports of work done on animals from Underhill and Ringel,⁹ Wallace and Pellm,¹⁰ Hashimoto,¹¹ Boyd,¹² and Miller,¹³ all agree on this. Small doses of histamine have been given human beings by Fonseca and de Carvalho,¹⁴ and Delhougne,¹⁵ who found an increase in plasma CO_2 in cases secreting hydrochloric acid in the gastric juice but not in achlorhydrias (achylas), by Katzenelbogen,¹⁶ who found an increase in plasma CO_2 in four cases of acidosis, and by Brunton and Israels,⁴ who reported a drop in alveolar CO_2 .

Feldberg and Schilf,¹⁷ in their review of blood and alveolar CO_2 changes produced by histamine have drawn the following conclusions, which we have freely translated. "The changes in alkali reserve and hydrogen ion concentration are proportional to the amount and dependent upon the method of injection. After small subcutaneous injections of histamine, there is an increase in the P_H of the blood. After intravenous or large subcutaneous injections, there is a large decrease of CO_2 tension and of P_H of the blood, this they attributed to the increased bicarbonate output in the urine, increased lactic acid production, increased pancreatic secretion, and the direct action of histamine."

*From the Chemical and Gastrointestinal Divisions of the Department of Medicine of Johns Hopkins University.
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TABLE I

CO VARIATIONS ESTIMATED ON ALVEOLAR AIR AND BLOOD PLASMA OR SERUM AFTER HISTAMINE INJECTIONS OF PATIENTS WHO SECRETED HYDROCHLORIC ACID INTO THE GASTRIC JUICE

NO	DIAGNOSIS	TIME MIN UTES	GASTRIC JUICE TITER %	BLOOD PLASMA OR SERUM		ALVEOLAR AIR CO ₂ CONTENT MM %
				CO ₂ CAPACITY VOLUMES %	CO ₂ CONTENT VOLUMES %	
6	Chronic cholecystitis	F *	18 31	57 9		
		30	77 87	57 9		
		60	97 108	56 0		
		90	64 73	58 3		
8	Cholecystitis	F	24 36	68 3		
		30	70 83	70 2		
		60	84 94	69 2		
		90	86 95	70 2		
10	Duodenal ulcer	F	2 18	52 2		
		30	61 71	70 2		
		60	62 72	70 2		
		90	0 10	68 3		
15	Diabetes mellitus	F	3 19	50 4		
		30	12 26	52 3		
		60	23 36	52 3		
		90		65 3		
38	Mitral regurgitation and functional dyspepsia	F	40 50	39 2		33 7
		30	23 35	42 0		37 5
		60	12 23	40 1		41 2
40	None	F	0 10	56 9		36 0
		30	24 36	56 9		40 5
		60	22 30	60 6		44 2
		90	20 32	60 0		40 5
42	Cholelithiasis and diabetes mellitus	F	40 52	62 6		38 2
		30	72 83	55 6		47 2
		60	76 84	57 9		50 2
43	Functional dyspepsia and hypertension	F	62 72	62 5		40 5
		30	74 84	62 5		43 5
		60	120 132	65 4		44 2
		90	120 127	65 0		44 2
44	Congenital constitutional inferior	F	0 10	63 5		42 0
		30	0 7	63 5		43 5
		60	50 58	62 8		42 0
45	Duodenal ulcer	F	32 40	59 6		46 5
		30	72 81	59 7		43 5
		60	72 80	59 7		45 0
		90	76 85			48 0
46	Chronic constipation	F	4 12	59 9		37 5
		30	68 74	62 3		43 5
		60	70 76	62 3		45 0
		90		62 0		45 0
50	Diabetes mellitus	F	0 8	50 4		36 0
		30	38-46	52 2		40 5
		60	28 36	54 1		44 2
		90	14-23	52 2		44 2

TABLE I (Cont'd)

NO	DIAGNOSIS	TIME MIN UTES	GASTRIC JUICE TITER %	BLOOD PLASMA OR SERUM		ALVEOLAR AIR CO ₂ CONTENT MM %
				CO ₂ CAPACITY VOLUMES %	CO ₂ CONTENT VOLUMES %	
52	Irritable colon and diarrhea	F	0 9	54 1		39 7
		30	20 30	59 8		41 2
		60	16 24	60 7		45 7
		90		61 7		46 5
53	None	F	0 8	53 8		38 2
		30	20 26	56 7		42 7
		60	22 32	57 6		44 2
		90		57 6		44 2
54	Hypertension and arteriosclerosis	F	78 86	56 0		33 0
		30	95 105	58 9		36 0
		60	121 129	59 8		39 7
		90	89 94	59 8		39 7
61	Chronic cholecystitis	F	0 6			31 5
		30	32 36			40 5
		60	10 15			39 0
		90	0 8			36 0
65	Functional dyspepsia	F	94 104	60 7		41 2
		30	93 104	70 1		42 0
		60	98 108	61 4		44 2
		90	68 80	60 4		46 5
71	Choleangectis secondary to cholecystoduodenostomy	F	6 23			24 7
		30	8 19			28 5
		60	62 72			30 0
73	Chronic appendicitis	F	0 8	58 6		
		30	86 95	57 6		
		60	108 118	61 4		
		90	88 98	62 4		
76	Cholecystitis	F	58 64			34 5
		30	6 14			35 2
		60	0 12			35 2
		90	20 30			33 7
84	Diabetes mellitus	F	0 12	57 9		
		30	29 41	59 7		
		60	32 46	67 1		
		90	0 14	66 4		
87	Chronic pancreatitis	F	0 10	56 7		
		30	18 30	63 3		
		60	38 48	59 5		
		90	32 42	60 5		
89	Irritable colon	F	0 10	63 6		
		30	30 40	63 6		
		60	44 56	60 3		
94	None	F	20 30	55 1		
		30	58 70	58 9		
		60	62 74	57 9		
		90	72 83	63 6		

TABLE I (Cont'd)

NO	DIAGNOSIS	TIME MIN UTES	GASTRIC JUICE TITER %	BLOOD PLASMA OR SERUM		ALVEOLAR AIR CO ₂ CONTENT MM %
				CO ₂ CAPACITY VOLUMES %	CO ₂ CONTENT VOLUMES %	
98	Fluorospasm	F	10 20	49 1	59 8	
		30	70 80	50 4	62 6	
		60	52 62	59 8	63 6	
		90	28 38	50 4	57 9	
99	Duodenal ulcer	F	42 52	54 1	60 7	
		30	87 97	57 9	63 6	
		60	94 104	57 0	63 6	
		90	92 100	59 8	62 6	
100	None	F	0 4	55 1	55 1	
		30	2 12	61 7	60 7	
		60	21 31	57 0	56 0	
		90	0 11	56 0	57 0	
101	Neurasthenia	F	12 26		56 0	
		30	36 48		61 7	
		60	48 54	61 7	60 7	
		90	50 57	60 7	59 8	
102	Chronic cervicitis, gall bladder disease ?	F	0 14	55 1	51 3	
		30	48 58	57 9	58 9	
		60	78 90			
		90	50 60	57 9	57 0	
103	Psychoneurosis	F	51 62	59 5	51 9	
		30	115 123	59 5	61 4	
		60	110 120	58 6	57 6	
		90	86 96	63 3	59 5	
104	Psychoneurosis	F	19 30	64 5	65 5	
		30	76 86	65 5	65 5	
		60	108 116	65 5	63 6	
		90	50 60	64 5	65 5	
105	Acne rosacea	F	0 10	62 4	60 5	
		30	38 48	67 2	66 4	
		60	52 63	65 5	63 6	
		90	60 70	63 6	61 7	

METHOD

In connection with the work done in this laboratory on phases of gastric digestion, we have made determinations of alveolar CO₂ and of plasma or serum CO₂ before and at half hour intervals after histamine stimulations of gastric secretions.

The patient, who had fasted for fifteen hours, reported to us in the morning. A duodenal tube was then swallowed and its position ascertained by the fluoroscope, with the tip in the most dependent portion of the stomach. The fasting contents were removed and control specimens of blood and alveolar air taken. Histamine was given subcutaneously (0.005 mg per pound of body weight) and at half hour intervals afterward specimens of blood and alveolar air were taken. The gastric juice was continuously aspirated and collected over the half hour

TABLE II

CO₂ VARIATIONS ESTIMATED ON ALVEOLAR AIR AND BLOOD PLASMA OR SERUM AFTER HISTAMINE INJECTION OF PATIENTS WHO SECRETED NO HYDROCHLORIC ACID INTO THE GASTRIC JUICE

NO	DIAGNOSIS	TIME MIN UTES	GASTRIC JUICE TITER %	BLOOD PLASMA OR SERUM		ALVEOLAR AIR CO ₂ CONTENT MM %
				CO ₂ CAPACITY VOLUMES %	CO ₂ CONTENT VOLUMES %	
51	Benign achylia	I *	0.4	54.1		41.2
		30	0.12	57.9		43.5
		60	0.11	60.7		44.2
		90		63.6		45.7
55	Carcinoma of stomach	I	0.8	47.5		33.0
		30	2.10	52.2		33.7
		60	0.7	58.9		36.7
		90				36.8
56	Chronic constipation	F	0.5	53.2		45.0
		30	0.8	54.1		45.7
		90	0.6	56.0		49.5
57	Ptosed, spastic colon	I	0.10			33.0
		30	0.10			34.5
		60	0.8			34.5
		90	0.10			35.3
58	Pernicious anemia	F	0.15			34.5
		30	0.16			35.5
		60	0.13			37.5
		90	0.12			37.5
58	Second test	F	0.10	57.9		35.5
		30	0.22	61.7		36.8
		60	0.22	61.7		39.0
		90	0.16	57.9		37.5
59	Pernicious anemia	I	0.18			36.0
		30	0.16			33.0
		60	0.14			36.8
		90	0.14			36.8
60	Chronic appendicitis and chronic cholecystitis	I	0.7	57.9		36.0
		30	0.13	57.9		37.5
		60	0.9	58.9		38.2
		90		60.7		38.2
62	Menopausal syndrome and chronic constipation	F	0.15	49.4		36.8
		30	7.17	52.2		39.0
		60		67.3		39.0
68	Chronic alcoholic gastritis	F	0.12	58.6		39.0
		30	0.10	52.8		38.2
		60	0.10	57.6		39.6
70	Pernicious anemia	F	0.4	60.7		34.5
		30	0.2	54.1		34.5
		60	0.2	59.8		35.5
		90	0.2	60.7		34.5
83	Chronic nephritis with anemia	F	0.16	43.8		
		30	0.14	49.4		
		60	0.13	40.9		
		90	0.12	40.0		

TABLE II (Cont'd)

NO	DIAGNOSIS	TIME MIN UTES	GASTRIC JUICE TITR %	BLOOD PLASMA OR SERUM		ALVEOLAR AIR CO ₂ CONTENT MM %
				CO ₂ CAPACITY VOLUMES %	CO ₂ CONTENT VOLUMES %	
88	Atonic colon	F	0 10	57 9		
		30	0 11	57 0		
		60	0 10	53 2		
		90	0 8	58 9		
90	Carcinoma of stomach	F	0 11	55 8		
		30	0 12	58 7		
		60	0 4	60 0		
		90	0 16	60 0		
92	Carcinoma of stomach	F	0 12			30 7
		30	0 28			31 5
		60	0 34			32 3
		90	0 10			30 7
92	Second test	F	0 6	56 0		
		30	0 50	54 1		
		60		57 0		
107	Cardiospasm	F	0 8	57 9	56 0	
		30		59 8	57 9	
		60	12 23	58 9	57 0	
		90	0 8	60 7	57 9	
108	Terminal nephritis	F	0 8	18 3	15 5	
		30		25 8	20 2	
		90		18 3	18 3	
109	Carcinoma of stomach	F	0 11		52 2	
		30	9 20		53 2	
		60	11 20		52 2	
		90	0 10		51 3	
110	Anemia pernicious?	F	0 13	60 7	59 8	
		30		59 8	57 0	
		60		58 9	58 9	
		90	0 10	59 8	57 0	
111	Carcinoma of stomach?	F	0 6	56 0	56 0	
		30	0 8	57 0	56 0	
		60		57 9	57 0	
		90	0 7	57 0	56 0	
112	Functional gastric disturbance	F	0 4	48 5	49 4	
		30	0 4	52 2	50 4	
		60	0 6			
		90	0 4	56 0	54 1	

periods Topfer's reagent was used in the estimation hydrochloric acid and phenolphthalein in that of total acidity. The plasma or serum were used interchangeably for the determination of CO₂ content and capacity, Van Slyke and Cullen's method¹⁸ was used. For alveolar CO₂ Frederica's indirect method¹⁹ was used.

OBSERVATIONS

We are able to report results of tests made on 52 patients, suffering from a multiplicity of ailments. A detailed list of these pathologic conditions is

given in Tables I and II. With but few exceptions all the individuals investigated were from the Gastrointestinal Dispensary of the Johns Hopkins Hospital and represent an average group of entrants. For clearness in the analysis of results we have divided the patients into two groups. Group 1 (see Table I) contains those secreting hydrochloric acid after histamine, this group also contains 14 patients who showed no hydrochloric acid under the stimulus of an Ewald test meal but who did secrete it after histamine. Group 2 (see Table II) contains those patients secreting no hydrochloric acid, the achylas who had gastric juice with a hydrogen ion concentration between 3 and 8.

In Group 1 there are 32 patients, 17 had hydrochloric acid in the fasting juice, the remainder did not. The type of CO_2 changes following histamine

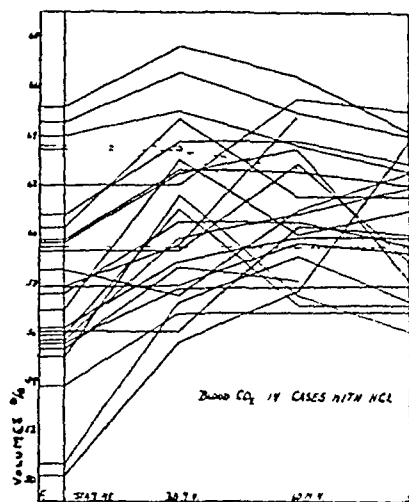


Fig 1

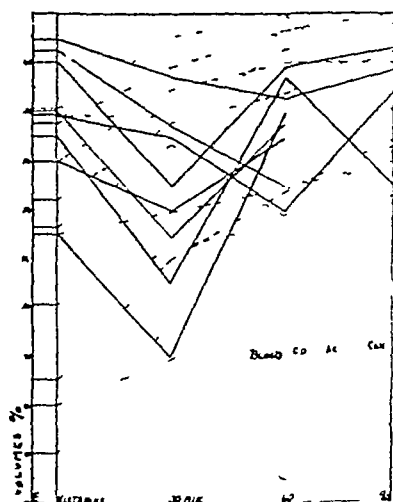


Fig 2

Fig 1—Changes in CO_2 capacity in blood serum or plasma after histamine stimulation of cases secreting hydrochloric acid in the gastric juice.

Fig 2—Changes in CO_2 capacity in blood serum or plasma after histamine stimulation of cases secreting no hydrochloric acid in the gastric juice.

did not seem to be influenced by the presence or absence of hydrochloric acid in the fasting juice.

A glance at Fig 1 demonstrates the fact that during the period of secretion, the majority of those patients secreting hydrochloric acid after histamine stimulation show varying amounts of plasma and alveolar CO_2 . In 25 patients there is an increase in plasma CO_2 and where the alveolar CO_2 was also determined there was a parallel increase. In two patients there was no demonstrable change, in two patients there was a fall. In three patients in whom the alveolar CO_2 alone was determined, there was a rise. There was no regularity in the time of the greatest increase but generally the peak was reached in thirty minutes, some cases continued to increase after the first rise.

In Fig 2 of Group 2, cases secreting no hydrochloric acid after histamine stimulation, the results are more irregular than in the previous group. The most marked point is that five patients out of the twenty show a definite drop in plasma CO_2 , paralleled by the alveolar CO_2 . The variations in those patients who

show a poststimulation rise in plasma and alveolar CO_2 are usually less than those which secrete hydrochloric acid in the fasting juice

Four cases of pernicious anemia are included in this group, in three there was a decrease in the alveolar or plasma CO_2 (in both if both were determined), but in the fourth there was an increase. The five cases of carcinoma show only small variations one way or the other. Two cases of terminal nephritis show an increase in the plasma CO_2 . One of these, No. 108, advanced from a control CO_2 capacity of 18.3 volumes per cent to 25.8 in thirty minutes, in ninety minutes there was a return to the control level.

DISCUSSION

When this work was started it was possible to assert that in patients secreting a normal gastric juice, the effect of a meal was to increase the alveolar CO_2 tension for various periods after ingestion. Furthermore one could add the evidence of one group⁹ of investigators that this rise was found only in cases secreting hydrochloric acid and not found in achylia. In their recent publication Peters and Van Slyke²⁰ present evidence which they feel corroborates this.

The consideration of the effects produced by histamine in human beings, however, does not allow one to make such sweeping statements as there is no agreement among the investigators. Perhaps the fact that but a small number of cases was studied by any group may be responsible for this lack of agreement.

From our work it is not possible to make any final statement as to the absolute certainty of a rise or fall in CO_2 of the plasma or alveolar air in relation to the type of gastric secretion. It is true, however, that the CO_2 of plasma and alveolar air always vary in a parallel manner.

The greater number of our acid secreting cases shows a rise in the CO_2 content but there are two definite exceptions. Furthermore, these exceptions secreted a large amount of juice of high titratable acidity. Our nonacid secreting cases do not all show a fall in their alveolar or plasma CO_2 after histamine stimulation. Some of them show a rising curve almost equal in height to that found in the acid secreting cases, others do not vary. Therefore, we cannot by any means foretell the CO_2 changes after histamine in patients not secreting an acid juice, the variation has an equal chance of being up or down.

The question as to whether the changes observed after a meal and those after histamine stimulation are comparable, is to be considered. Certainly the main action of food in normal cases is the production of hydrochloric acid. It is possible that as pancreatic secretion begins, this secondary action may greatly influence the electrolytic balance in the blood. With histamine and with the constant evacuation of the gastric contents, it is possible to lessen the degree of the second factor. Nevertheless in the majority of our cases we find the same results as those reported in all acid secreting cases after eating a normal meal, i. e., an elevation of alveolar CO_2 . Therefore, although the fact that histamine may have other indirect effects upon the alveolar and the plasma CO_2 than that produced by gastric secretion it appears that in the large part, the changes are those that follow this secretion.

CONCLUSIONS

1 Following the stimulation of gastric secretion by histamine, there is an increase in the CO_2 content of alveolar air and of plasma or serum of the great majority of patients who secrete an acid juice

2 Following the stimulation of gastric secretion by histamine, there may be an increase or a decrease in the CO_2 content of alveolar air and of plasma or serum of patients who secrete no hydrochloric acid in the gastric juice

3 The variations in the CO_2 content of alveolar air and of plasma or serum parallel each other after histamine stimulation of gastric secretion

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INTRADERMAL TEST FOR THE DETERMINATION OF MALIGNANCY*

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THE object of this paper is to present a new phase in the diagnosis of malignancy, with an attempt to establish the fact that a foreign protein of embryonal character is present in malignancy and that this protein is so characteristic that it not only produces specific precipitins, as demonstrated by the serologic test for malignancy,¹ but also shows that the fixed cells, too, are responsive to that protein, which fact is demonstrated by the allergic reaction obtained when an antigen of embryonic tissue is used. It further proves that these embryonic proteins are distinctly of a given type, that is, they are either epithelial in the case of carcinoma or of connective tissue origin in the case of sarcoma.

Long ago, many workers noticed the embryonic character of malignant cells and have even attempted to make extracts of embryos for therapeutic purposes but failed to appreciate the fact that while it is true that malignant cells are embryonic in character, the great difference lies in the type of the cell which determines its specificity, this, they have entirely overlooked. That is to say that carcinoma, being an epithelial tumor, will not respond to embryonic tissue which contains connective tissue cells, and vice versa in the case of sarcoma.

Fly² employed tumor tissue as an antigen for a serologic test for malignancy and, according to his own statement, the test did not prove successful in a great number of cases. The reason for it may be ascribed to the fact that tumor tissue can not be obtained in pure form to represent a given type of cell, on account of the intercellular substance and mature connective tissue cells which surround the malignant cells and which cannot be separated from them in the preparation of the antigen. Furthermore, if a malignant tumor is large enough to be used for antigenic purposes, degeneration of the tumor tissue is usually already in progress and will interfere with the reaction.

One must also keep in mind the possibility of organ specificity, as proved by Hektoen³ and his co-workers, which is liable to give false reactions in allergically susceptible individuals. From our observations in the Clinic here at the University, a great number of patients suffering with malignant conditions have shown allergic phenomena to other proteins too. It is therefore imperative that the antigen chosen for the reaction must be of such nature that is highly allergically specialized and not within the category of nonspecific allergic susceptibilities.

Worthy of consideration is the possibility that inoculation of a patient with an antigen made of cancer tissue, enough to produce an allergic reaction, might exert antigenic influences in encouraging malignancy in an inherently susceptible

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individual. The fact that implantation of cancer tissue in man does not develop malignancy does not prove contrary to the above possibility for the simple reason that when cancer is implanted, degeneration of the implant rapidly takes place on account of the lack of blood supply, and therefore the antigenic influence does not assert itself, while when an extract of purely epithelial cells is introduced, then characteristic protein might act immediately, as demonstrated by the allergic reaction.

As seen from the above, the preparation of an antigen for the purpose of determining malignancy must be of such nature that it will not be affected or marred by any of the above enumerated obstacles. In order to do so, cells of purely embryonic character must be resorted to, and accordingly the following tissues have been adopted and found to give the desired results. In the case of carcinoma, pancreas and submaxillary glands of very young embryo calves were used, for sarcoma, Wharton's jelly and the bone marrow of young embryos were utilized, as per the following technique.

Preparation of Antigen, Preliminary Steps—It is essential that in the preparation of the antigen, both for carcinoma and sarcoma, all glassware and other utensils used be chemically clean, sterile, and of neutral reaction.

Preparation of the Antigen for Carcinoma—Mammalian embryos are used (calves, sheep, pigs). They must not be in a later stage than the second month of fetal life. This is readily recognized by their relative smallness, and by the smoothness of the skin (for instance, there is no formation of hair). In securing these embryos, the abdomen must be opened under sterile conditions. The pancreas and submaxillary glands are dissected out, and placed in a sterile dish. A hypotonic salt solution is poured over these, and if possible, allowed to freeze, the object being to permit an easier removal of the fibrous capsule. The capsule and the ducts, etc., are then removed by careful manipulation with small tissue forceps. The epithelial tissue is picked out. It is placed in a mortar in which sterile copper gauze is inserted (to facilitate the maceration) and macerated in physiologic salt solution. At this point, the cells suspended in salt solution are centrifuged and the supernatant salt solution is discarded. The epithelial cells are placed in a porcelain dish and dried at a temperature of 75°C for a few minutes until the water separates, which should then be poured off leaving the cells in a state of doughy consistency. These are then covered with ether, shaken up, the ether decanted and the cells placed at room temperature for a few minutes to allow the remaining ether to evaporate. It is then rubbed up with twenty-five times its volume of one-tenth normal sodium hydroxide, by adding the sodium hydroxide slowly, one cubic centimeter at a time, and macerating it vigorously until the cells are thoroughly rubbed up, so that on standing for awhile the least amount of the remaining cells fall to the bottom. It is placed at room temperature for twenty-four hours, after which it is centrifuged at a low speed for ten minutes, the supernatant fluid is then pipetted off and neutralized with one-tenth normal hydrochloric acid, drop by drop, until it is brought to neutrality, at a pH of seven.

Recent experiments have shown that embryonic liver may be used to advantage in the preparation of the antigen for carcinoma, since a larger amount

TABLE I

CASE	GRUSKIN INTRADERMAL	BIOPSY OR AUTOPSY	OPERATION	CLINICALLY OUTSPOKEN
H F	Carcinoma positive	Carcinoma of breast	Carcinoma of breast	
B K	Carcinoma negative			Advanced, active bi lateral pulmonary tuberculosis
C J	Carcinoma positive	Carcinoma, probably bronchiogenic	Carcinoma of larynx	
R S	Carcinoma positive	Extensive adenocar cinoma	Adenoma of ovary	
J R	Carcinoma positive	Fibroadenoma, ma lignant change		
M P	Carcinoma negative			Advanced pulmo nary tuberculosis, laryngeal tubercu losis
M C	Carcinoma positive	Chronic cystic mas titis		
E B	Carcinoma negative	Chronic cystic mas titis	Chronic cystic mas titis	
E C	Carcinoma negative			Syphilis
Mrs D	Carcinoma positive			Confirmed by x ray
F T	Carcinoma negative			Bilateral pulmonary tuberculosis
F H	Sarcoma positive	Endothelioma of bone that has spread to sur rounding soft structures		
V B	Sarcoma positive	Melanosarcoma	Melanotic sarcoma	
P W	Carcinoma negative			Diabetes
J L	Carcinoma positive	Fibroadenoma un dergoing malign ant change	Fibroadenoma of breast	
V B	Carcinoma negative			Incipient tubercu losis
R W	Carcinoma negative			Nephritis
H P	Carcinoma positive			Carcinoma of pros tate, with metas tasis to lungs and bones
A B	Carcinoma negative			Syphilis
T W	Carcinoma positive	Alveolar carcinoma of appendix	Acute appendicitis with pelvic ab scess	
L A	Carcinoma negative			Early pulmonary tu berculosis

TABLE I (Continued)

CASE	GRUSKIN INTRADERMAL	BIOPSY OF AUTOPSY	OPERATION	CLINICALLY OUTSPOKEN
S P	Carcinoma negative		Abcess in tempero mandibular region	
M A	Carcinoma positive	Squamous cell car cinoma	Carcinoma of cervix	
R W	Carcinoma negative			Early tuberculosis
W S	Carcinoma positive			Carcinoma of pelvic organs with me tastasis to peri toncum
L S	Carcinoma negative			Syphilis
E L	Sarcoma positive	Periosteal sarcoma	Sarcoma of iliac bone	
H M	Carcinoma positive	Adenoma of stomach undergoing malig nant change	Gastric malignancy	
L M	Carcinoma negative			Fibroid tuberculosis
J W	Carcinoma positive	Carcinoma		
R J	Carcinoma positive	Adenoma undergo ing malignant de generation	Carcinoma of gall bladder	
L B	Carcinoma positive	Necrotic tissue, ma lignant	Carcinoma of cervix	
E G	Carcinoma negative			Bilateral pulmonary tuberculosis
R M	Carcinoma negative	Early Hodgkin's disease		
C E	Carcinoma positive			Extensive carcinoma of bladder (mili trating)
A S	Carcinoma negative			X ray tuberculosis of lung
M B	Sarcoma positive	Retroperitoneal sar coma		
E H	Carcinoma negative			Active pulmonary tuberculosis
B E	Carcinoma positive	Suspicious malig nancy	Possible carcinoma of vagina	
B G	Carcinoma positive		Considered inoper able	Carcinoma of breast
A B	Carcinoma negative			Bilateral pulmonary tuberculosis
S A	Carcinoma positive		Carcinoma of pros tate	
H G	Carcinoma negative			Diabetes

TABLE I (Continued)

CASE	GRUSKIN INTRADERMAL	BIOPSY OR AUTOPSY	OPERATION	CLINICALLY OUTSPOKEN
E C	Carcinoma negative	Chronic interstitial adenitis		
F T	Carcinoma positive	Epithelioma		
K H	Carcinoma negative			Bilateral pulmonary tuberculosis
J L	Carcinoma positive	Labial carcinoma	Labial carcinoma	
S R	Carcinoma positive	Metastatic carcinoma		
A B	Carcinoma positive		Carcinoma of cecum and sigmoid	
M B	Carcinoma negative		Lung abscess	
L K	Carcinoma negative			Pneumothorax case, chronic prostatitis, duodenal ulcer
A H	Sarcoma positive, Carcinoma negative	Spindle cell sarcoma, Hodgkin's disease		
H M	Carcinoma negative			Syphilis
J A	Carcinoma positive	Squamous cell carcinoma	Necrosis external ear, unhealed radical mastoid	
S G	Carcinoma negative	Simple colloid goiter with considerable fibrosis	Adenoma of thyroid	
J M	Carcinoma positive	Rodent ulcer		
Mrs H	Carcinoma positive	Mucinous carcinoma, cervical endometrium		
Mrs C	Carcinoma positive	Adenocarcinoma of rectum, resected		
Mrs M	Carcinoma positive	Squamous cell carcinoma of the forehead Diabetic		
Mr McC	Carcinoma positive	Teratoma (mixed carcinoma sarcoma) mucinous type		
Mr G	Carcinoma positive	Adenocarcinoma of the prostate		

Twenty cases of normal individuals gave negative reactions

Four cases of diabetes gave negative reactions

One case of polycythemia gave a negative reaction

of material may be obtained with greater ease. From the embryonic liver the cells are obtained in the following manner: the whole liver is cleaned of the capsule and washed with water to remove as much blood as possible. It is then placed in an Erlenmeyer flask with water and shaken vigorously until all the cells

are separated from the fibrous tissue. The cells and water are then centrifuged at high speed for five minutes, the water poured off and the cells washed repeatedly until all traces of blood are removed. For the intradermal antigen the wet cells are rubbed up with twenty volumes of one-tenth normal sodium hydroxide, allowed to stand for twenty-four hours, and then neutralized and prepared as in the case of the embryonic pancreas. For the serum antigen, the cells are placed in five volumes of acetone for twenty-four hours, then centrifuged, the acetone poured off and the cells dried in a desiccator. They are then rubbed to an impalpable powder and mixed with alcohol as described in the case of the embryonic pancreas.

Antigen for Sarcoma—A mixture of Wharton's jelly and red bone marrow of calf embryo is macerated in a mortar, to which two volumes of salt solution are added. This is placed in a porcelain dish at a temperature of 75°C until the water separates, which should then be poured off, leaving the cells in a state of doughy consistency. These are then covered with ether, shaken up, the ether decanted and the cells are placed at room temperature for a few minutes to allow the remaining ether to evaporate. It is then rubbed up and with one tenth normal sodium hydroxide, in the proportion of one gram of cells to 25 c.c. of sodium hydroxide. The sodium hydroxide is added slowly, one cubic centimeter at a time, macerating the cells vigorously until they are thoroughly rubbed up, so that on standing for awhile the least amount of the remaining cells fall to the bottom of the tube. The tube containing the solution is placed in the ice box for twenty-four hours after which the supernatant fluid is pipetted off and neutralized with one-tenth normal hydrochloric acid, added drop by drop until it is brought to neutrality, at a P_{H} of seven.

After being neutralized, the antigens are then placed in small vaccine bottles and kept in a cool, dark room and are ready for use.

The Test Proper—Two tenths of a cubic centimeter of the antigen is injected intradermally with a very fine needle. Care must be taken that the injection should not be forced, so that no false pseudopods will be formed. In positive cases, a slight area of inflammation with pseudopod formation appears within fifteen minutes. In negative cases, no such reaction takes place. It is advisable to use a control of plain physiologic salt solution with each test. The control must always be negative, showing no inflammation and no pseudopods.

Precautions—In patients who are emaciated and where the skin assumes a paper-like thinness so that a correct intradermal test is impossible to be performed, it is advisable to resort to the serologic test described by the author in the *American Journal of the Medical Sciences*, April, 1929. For in these cases on account of the irregularity in the contour of the skin, one might mistake the natural appearance for pseudopods, and, vice versa, the pseudopods might not be easily distinguished. It is also advisable not to perform the intradermal test on patients with septic temperatures or jaundice, nor soon after x-ray, radium treatment, or anesthesia, as false reactions might take place in such cases.

Summary—An intradermal test for the determination of malignancy has been introduced. The antigen is made up of purely embryonic tissue obtained from pancreas and submaxillary glands of embryonic calves, in the case of carcinoma, and of Wharton's jelly and red bone marrow in the case of sarcoma.

The theory is advanced that the characteristic embryonic protein is carried not only in the blood stream but also finds response in the fixed cells, as expressed by the allergic reaction. The correct results obtained in a great number of positive and negative cases have been demonstrated, so that we feel justified in publishing this preliminary report. It is of interest that in 116 cases of intradermal tests done on students under the auspices of Professor Fanz, head of the Department of Pathology of Temple University School of Medicine, the following results were obtained:

One hundred and seven students gave no reaction.

Eight students gave a slight reaction to carcinoma, and of these, the following history was obtained: 1 had a maternal history of malignancy for three generations, 1 had a maternal history of malignancy for two generations, 6 had a family history of malignancy for one generation.

One student gave a slight reaction to sarcoma and no reaction to carcinoma. He had a paternal history of sarcoma.

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LABORATORY METHODS

TECHNICAL CONTRIBUTIONS TO THE STUDY OF GASTROINTESTINAL MOTILITY*

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I ARRANGEMENT AND ADJUSTMENT OF MANOMETERS

THE application of the tandem-balloon method, with the use of large numbers of balloons so placed as to correlate the activity of different levels of the gut in a serial manner, has been limited by difficulties which are self-evident. When water manometers are used as recording apparatus, the problem is presented of arranging them so that their writing points are in vertical alignment. To overcome this difficulty a multiple manometer clamp has been devised which allows the entire set of manometers to be moved as a unit without disturbing the relationship of the manometers to each other and which allows larger numbers of manometers to be clamped in a small space than is possible with other methods (Fig 1-1)

Individual manometers can be raised or lowered or removed from the clamp without disturbing the others. In order to remove a manometer, it is necessary to lift off the cap and the writing arm, after which the manometer can be drawn downward by releasing the screw which clamps it. It is advisable in setting up manometers in the clamp to place those which will write on a lower level on the side of the clamp next to the recording surface. In this way there is less conflict between writing arms as they pass to the drum. The clamp is conveniently set with its long axis at an angle of 20° to 30° to the recording surface.

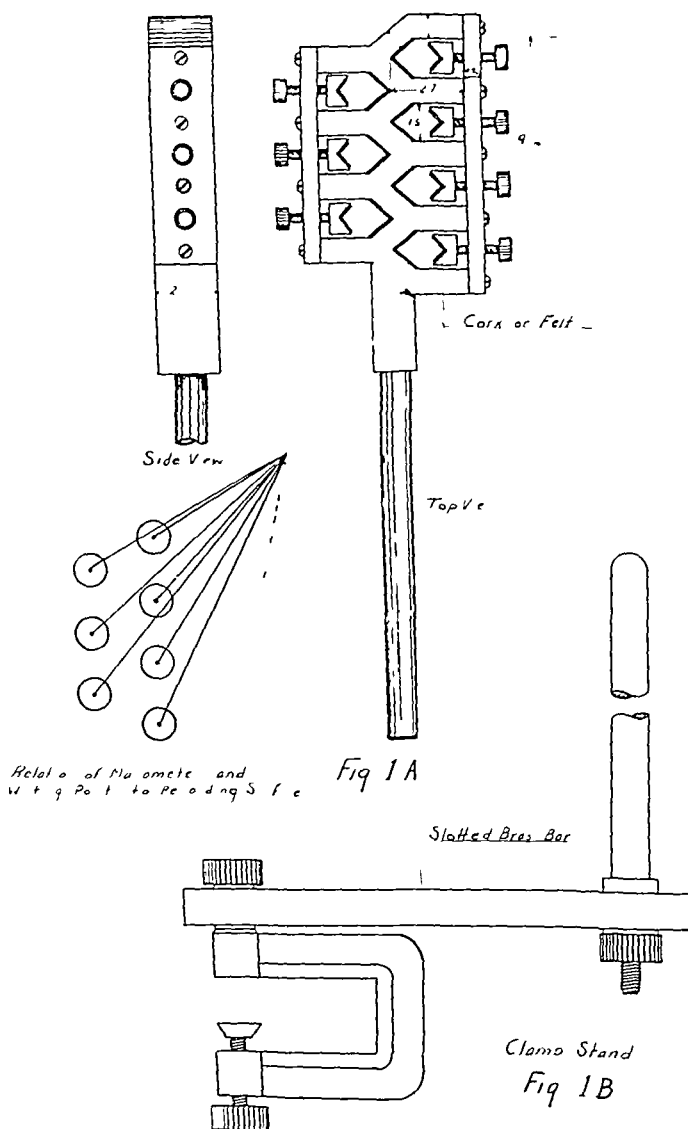
A clamp stand was constructed for holding the manometer clamp, by bolting a rod into a slotted bar so that the rod could be moved in the slot, and brought into any desired position. The slotted bar was in turn bolted into an ordinary wood clamp, so as to rotate about its point of fixation to the latter (Fig 1-B). The wood clamp is fastened to the table or kymograph stand, and the entire battery of manometers can be swung into or out of position.

With this arrangement of manometers the usual methods of holding the writing arms against the drum are impracticable. A simple guard was formed by gluing in a vertical position from the manometer cap a flexible strip of celluloid which could be brought to bear suitable pressure against the writing arm. A more permanent and efficient arrangement is now in use (Fig 2). A drawn glass tube is fastened into a coiled spring of just sufficient strength

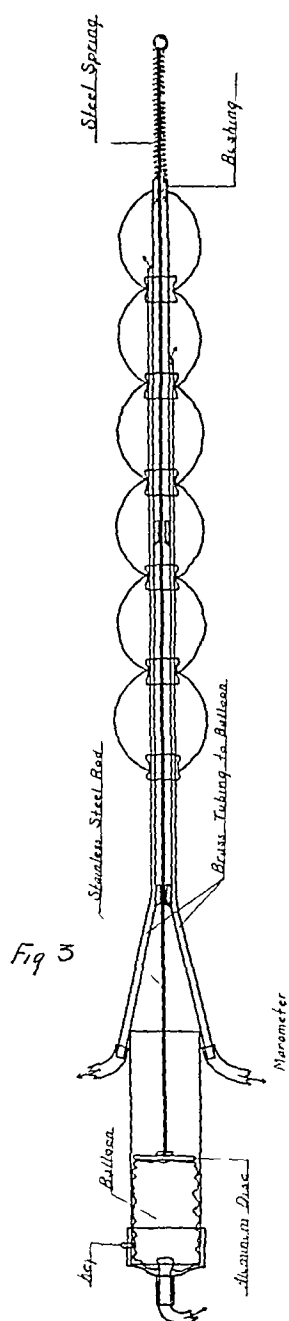
*From the Physiological Laboratories of the University of Chicago and the Department of Physiology of Loyola University School of Medicine
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to support the glass tube vertically. The spring is soldered to the side of the manometer cap. The guard is brought into position against the writing arm by rotating the cap. To prevent rocking of the cap, with disturbance of the guard, the sides of the cap are made much longer than is customary. The

Multiple Manometer Clamp



caps now in use are 4 cm tall. To prevent rotation of the cap, with change of tension against the writing arm, a thin sheet of phosphor-bronze was bent at angles as shown in the cross section, and inserted within the cap before forcing the latter on the manometer head. Caps were made to fit the largest manometer



The plunger itself is constructed of stainless steel about 2 mm in diameter of cross section, and is made sufficiently longer than its protecting tube to record the expected activity. The diameter of the apparatus is kept as small as possible, by using small tubes, and by flattening the balloon leads against the plunger tube. The balloon leads run under collars which afford a smooth surface for tying balloons. Solder is run in under the collars to make an airtight seal between balloon compartments.

The end of the plunger which impinges against the wall of the gut is capped with a small metal ball. A coil spring is compressed between this ball and the end of the plunger tube. The spring is of sufficient strength to hold the plunger fully extended when outside the gut and is not fastened at either end. When the balloon is distended within its cylinder, the spring may be grasped between two fingers and completely compressed against the end of the plunger tube without moving the plunger.

A number of technical difficulties are encountered in using this apparatus. When the plunger becomes inactive during a tracing, after progressive lowering of the writing level the explanation may be found in a leak in the balloon lying within the cylinder which has caused the balloon to collapse away from the piston. On the other hand there may have been a relaxation of the gut so that the force applied against the end of the plunger is no longer sufficient to move it. The tracing does not differentiate between the two situations. However, by cutting away completely, or slotting the plunger tube as it enters the cylinder, the plunger is rendered visible, as shown in the sketch and the position of a scratch on the plunger is a valuable guide. If the pouch has relaxed the plunger will be found completely extended, if the balloon has leaked the plunger may be in its original position. To allow for moderate relaxation of the pouch, the apparatus is inserted so that the plunger is about half extended at the start.

The coil spring which is slipped over the extended portion of the plunger serves two purposes one of which is theoretical. The spring serves to keep the plunger extended, allowing the balloon within the cylinder to be distended under lower pressure. It was also thought that it might protect the plunger from movement under the influence of such activity as peristalsis which could conceivably be of such intensity that the moving ring of constriction would grasp the unprotected plunger and sweep it downward. It was thought that the coils of the spring would be moved instead, if this occurred and plunger movement diminished.

For use in the exposed intestine a perforated ball or a small metal ring is attached to the extended end of the plunger by means of which the plunger is anchored in place with a stitch carried through the intestinal wall. This obviates the necessity of preparing an intestinal pouch for acute work.

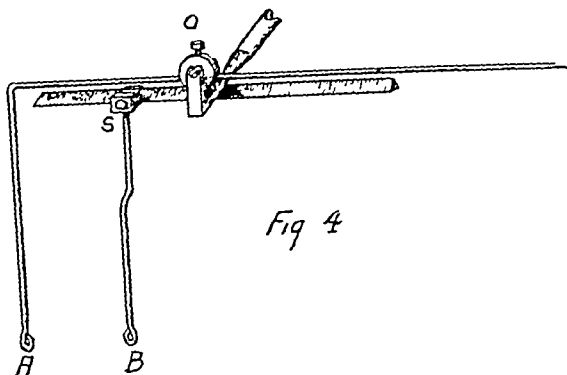
In inserting the apparatus the cap and the balloon are removed from the cylinder. When the plunger is in position against the end of the pouch, the balloon is inserted within the cylinder the cap slipped on, and all balloons inflated. In Fig 3 six tandem balloons are shown. The sketch has been simplified by omitting the side tubes leading to all but two of these. With this number of balloons almost any length of pouch can be studied without recon-

struction of the apparatus. Most of our pouches are of such length that only three or four of the tandem balloons lie in the pouch when the plunger is inserted to recording position.

In order to minimize mechanical errors due to lateral movements of the animal during the course of a tracing, the apparatus is held in position by means of a clamp made from a large burette clamp by placing two swivel joints, with their axes of rotation at right angles, in the arm of the clamp. The apparatus thus rotates freely about a fixed point, and the axis of the plunger is automatically aligned with the long axis of the pouch. The clamp is applied to the cylinder, and secured in an ordinary clamp stand which is bolted to the table.

C. A SIMPLIFIED ENTIROGRAPH

The apparatus differs from a number of devices designed for the same purpose in its simplicity and its adjustability. The materials required are a frog lever and some stiff wire. One length of wire is passed through the pivot of the frog lever, and fastened in place by the set screw, after being bent at right



angles. Another, shorter piece, is soldered to the fulcrum of the lever. The ends of the wire are bent to form eyes for fastening to the intestinal strip or ring. The tracing may be taken directly from the end of the long arm of the movable lever, or transmitted to a second lever for recording.

A modification of this apparatus which has the advantage of wide adjustability without change of leverage is shown in Fig 4. The distance between the movable end *A* and the fulcrum *O* may be kept constant while adjustments are made by shifting the position of the passive arm *B* on the cross piece. The sleeve *S* which carries this arm is open above so that it slides freely over the entire length of the cross piece. The latter is soldered to the fulcrum of the frog lever at such a point that the cross piece and the movable arm of the lever do not lie in a vertical plane. If this is not done the lever and the cross piece will be brought into contact with high activity. The arms *A* and *B* are made sufficiently long to carry the gut, in position between them, below the surface of the bath. In working on large numbers of segments, or on the exposed gut in situ, it is usually more convenient to transmit the movements of the lever to a second lever by means of a string for taking the record.

The writers wish to express their gratitude to Mr Gus Lutz, of the Mechanical Department, whose practical suggestions were invaluable.

FRAGILITY OF RED CORPUSCLES AND ITS DETERMINATION IN CLINICAL WORK²

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INTRODUCTION

RED corpuscles mixed with water or hypotonic solutions disintegrate and show hemolysis. Experiments made during the last years proved that the old opinion which attributed the cause of this process to some changes of the membrane of corpuscles is untenable. The pellicle is permeable to hemoglobin and can disappear without causing a hemolysis. Hemoglobin is probably combined with some substances in the corpuscles chemically, and this compound is decomposed by all agents producing hemolysis. The case with which the disintegration of the protoplasm of red cells takes place may be called their fragility.

Hamburger, in his recent essay concerning the methods for the determination of the resistance of red corpuscles to hypotony,¹ came to the conclusion that the clinical application of these methods was not successful. Such a conclusion is not surprising because the methods used were either too complex or gave inexact and incomparable results. In most cases a threshold of hemolysis that is a concentration in which no hemolysis is observed, but a slight decrease of which leads to hemolysis, was determined. Hamburger pointed out rightly that the results of this method depended upon the volume of the solutions and the size of containers used. Moreover, traces of hemoglobin in the solution were discovered with a different accuracy by different observers. To these two objections we may add two more.

The determination of the threshold of hemolysis gave only an account of the state of the corpuscles with the greatest fragility. A change of conditions however could influence the corpuscles with a median fragility more than other corpuscles. Sometimes the threshold of hemolysis is found alike in different men, whereas the fragility of their red blood cells with median properties differs by more than 100 per cent (see below). Moreover in all methods used up to the present only the volume of the corpuscles and not their hemoglobin content was considered. As the water content of the corpuscles may vary, the hemolysis may be found alike whereas the fragility may be different.

Among the methods used for the determination of the resistance of corpuscles to hypotony Aikhenius' method is the only one which does not depend upon the volume of solutions and containers and permits to observe the changes in corpuscles of different fragility. The author had the opportunity to work in Aikhenius' laboratory and to use his method in most experiments. The application of this method to clinical work requires, however, some improvements

¹From the Laboratories of the Desert Sanatorium and Institute of Research, Tucson Arizona.
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First, one has to avoid taking too much blood from a patient. Second, a prolonged centrifugalization before testing should be avoided, because every centrifugalization decreases the resistance of corpuscles to hypotonic solutions.² Third, it is well known that salt solutions change the fragility of corpuscles, therefore, one has to avoid a too prolonged action of any salt on corpuscles before they are put in a hypotonic solution. Fourth, not the volume of corpuscles but their hemoglobin content should be considered in estimating the number of hemolyzed corpuscles. All these requirements are fulfilled in the following method used by the author in his experiments on the influence of sunlight on men.

METHOD

Hypotonic Solutions—To avoid coagulation of blood hypotonic solutions of sodium sulphate are used. Four solutions are prepared for a whole series of experiments at once in the amount of 500 c c by diluting a standard solution with distilled water. The standard solution contains 16.77 gm of anhydrous sodium sulphate in 1000 c c. To both, water and the standard solution, a sufficient amount of the solutions of monobasic (1.5 per cent) and dibasic sodium phosphate (3.5 per cent) is added to adjust P_H to 7.3. The solutions are prepared by mixing the following volumes of the standard solution with water.

	STANDARD SOLUTION	DISTILLED WATER
First sol (0.70 per cent)	209.5 c c	290.5 c c
Second sol (0.64 per cent)	191.5 c c	308.5 c c
Third sol (0.60 per cent)	179.5 c c	320.5 c c
Fourth sol (0.54 per cent)	164.5 c c	335.5 c c

The solutions are kept in glass stoppered bottles in a refrigerator, and once in two weeks the P_H of the solutions is tested and adjusted if necessary to 7.3. With a pipette, 5 c c of each solution are poured into four centrifuge tubes, closed with corks. Into the fifth tube (also closed with a cork) 5 c c of distilled water are poured. All solutions should have the temperature of the room before adding blood.

Blood is taken either from a finger or from the cubital vein. In the first case one Sahli pipette full of blood (0.02 c c), in the second case one drop of blood, is introduced into each of the 5 centrifuge tubes, and the latter are shaken once and left standing for half an hour (to settle the osmotic equilibrium), and then shaken once more and centrifuged for two minutes to sediment the corpuscles.

The estimation of the degree of hemolysis produced by each solution may be made by means of a colorimeter comparing the color of the supernatant liquids from tubes 1 to 4 with the color of the liquid in the fifth tube. This method is however not exact in our case because of the small amount of liquids and of too great a difference between the colors of the liquids. More accurate is the following method used by the author which does not require any expensive colorimeter.

This method rests on the same principle as the Sahli method for the estimation of hemoglobin. The color of the supernatant liquids in the second to fourth tubes is compared with the color of the properly diluted completely hemolyzed solution in the fifth tube. After the dilution this solution should have a fainter tint than the solutions to be estimated. This is achieved by diluting it in the following way.

For fourth and third solutions 4 cc of solution 5 plus 16 cc distilled water. This diluted solution is diluted further.

For third and second solutions 5 cc of it plus 5 cc water.

For second solution* 5 cc of it plus 15 cc water.

After the dilution is made, exactly 2 cc of the supernatant liquids from one of the solutions to be estimated (second to fourth) are poured into a specimen vial (5 cm high, 2.5 cm wide) graduated in cubic centimeters with divisions of 0.2 cm. Into a similar vial which needs no graduation, the corresponding diluted solution is poured. The height of the liquids in the vials should be the same so that the amount of the liquid in the not graduated vial should be adjusted to the amount in the graduated one. Both vials are standing on a white porcelain plate (6 inches square) at the posterior edge of which a mirror (the same size) is fastened which reflects light from the window onto the vials (the observer is sitting with his back to the window). Then by means of a pipette with a rubber ball water is added by drops to the solution to be tested until the color of the liquids in both vials becomes equal. Not only the color of the column of the liquids but also that of their menisci is considered. The height of the meniscus in the graduated vial after the color of the liquids has been considered equal, expressed in cubic centimeters (according to the graduation) and multiplied by 10 represents the percentage of hemolysis in case the fourth solution is tested. Multiplied by 10 or 5 (according to the dilution used) it represents the percentage of hemolysis in the third solution, and multiplied by 5 or two and a half the percentage in the second solution.

The hemolysis in the first solution is very slight, and it should be estimated in the following manner:

Into one specimen vial, 4 cc of this solution, into the other vial 4 cc of water are poured, and then by means of a pipette, 1 cc divided into 0.01 cc, the completely hemolyzed solution from the fifth tube (1 cc left from the dilution) is added to water, until the color of the liquids and their menisci in both vials becomes equal. The corresponding percentage of hemolysis is the following:

The amount of solution from the fifth tube added in cc	0.01	0.02	0.04	0.05	0.12	0.16	0.20	0.24
Hemolysis %	0.2	0.5	1	2	2.9	3.8	4.8	5.7

RESULTS

Average Fragility—Supposing that the hemolysis, in percentage, is found in the first solution 12, that in the second solution 11, that in the third 26 and that in the fourth solution 62, the average fragility of red cells is 25. It expresses the fragility not only for a certain kind of cells, that is, for the most resistant or for the most fragile ones but the average of all cells.

In Table I the hemolysis in the above solutions and the average fragility is given for a normal man, in Table II for different men at 11 and in December 1931 (Tucson, Arizona).

From Tables I and II it may be concluded that the estimation of hemolysis produced by one hypotonic concentration, for instance the determination of a threshold of hemolysis, does not give us an idea about the average fragility of

*The second and third solutions may be therefore compared twice: first with a less and second with a more diluted solution 5.

TABLE I
NUMBER OF HEMOLYZED CELLS IN PERCENTAGE FOR A NORMAL MAN

DATE, TIME	NA ₂ SO ₃	0.70%	0.64%	0.60%	0.54%	AVERAGE FRAGILITY
3 P.M. Dec. 15, 1930		2.5	19	40	85	36.6
3 P.M. Dec. 17, 1930		2.5	23	60	85	42.6
11 30 A.M. Dec. 19, 1930		2.7	28	51	87	42.1
11 30 A.M. Jan. 7, 1931		4	27	58	90	44.7
11 A.M. Feb. 19, 1931		1.7	12	32	73	29.7
10 A.M. March 11, 1931		2.5	11	36	88	34.3

TABLE II
NUMBER OF HEMOLYZED CELLS IN PERCENTAGE FOR DIFFERENT NORMAL MEN

AGE	NA ₂ SO ₃	0.70%	0.64%	0.60%	0.54%	AVERAGE FRAGILITY
25	3	28	64	95		47.5
25	2	21	64	92		44.7
25	2.5	26	52	86		41.6
25	1.8	22	54	92		42.4
28	2.5	23	58	93		44.1
28	2.5	23	60	86		42.9
30	3	28	64	94		47.2
31	2	30	60	83		43.7
35	1.8	20	59	96		44.2
35	1.8	23	49	82		38.9
42	2	21	48	82		38.2
43	2	26	49	74		37.7
50	1.8	20	40	76		34.4
55	2.2	22	50	65		34.8
60	3	27	54	62		34

Average for the age 25 to 40 2.3 24.4 57.4 89.9 43.7

Average for the age 40 to 60 2.2 23.5 48.2 71.8 35.8

The average fragility of red cells for all ages is about 40, its maximum is 47.5 and its minimum is 34

TABLE III
NUMBER OF HEMOLYZED CELLS IN PERCENTAGE

EXPERIM NO	FRAGILITY DETERMINED	NA ₂ SO ₃	0.70%	0.64%	0.60%	0.54%	AVERAGE FRAGILITY
1	Before the exposure to sunlight		2.7	28	51	87	42.1
	After the exposure for half an hour		0.9	11	36	75	30.7
2	Before rise of temperature		2.5	15	36	73	31.6
	After the maximum temperature was reached		1	5	17	40	15.7

red cells. The hemolysis in one concentration can be alike, while the average fragility may differ quite distinctly. From Table II it is even seen that the degree of hemolysis in small concentrations, and therefore the threshold of hemolysis, is often greater in some men than in others, while this degree in stronger concentrations, and also the average fragility are greater in the former than in the latter. It is evident therefore that only the whole curve of hemolysis can give us a correct idea about the changes of the fragility of red cells. To obtain the curves we may plot on the abscis axis the concentration of Na_2SO_4 and on the ordinate axis the hemolysis in percentage. The variation of fragility in a single man is expressed in the curves of Fig 1.

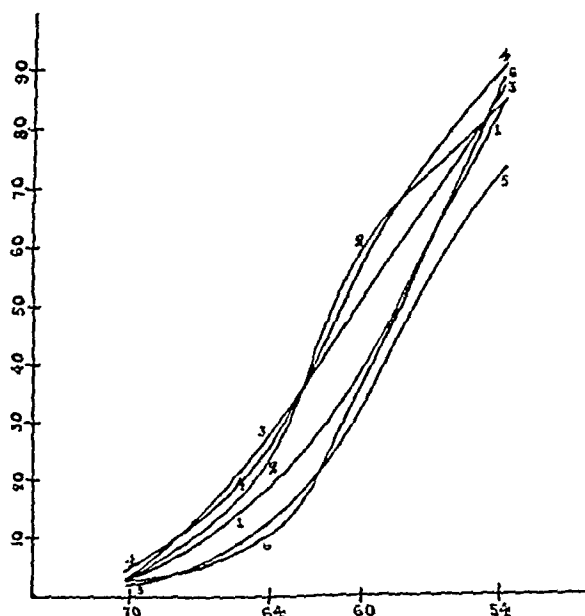


Fig 1—The curves of fragility of red corpuscles in a normal man 1, 3 P M Dec 15 1930 2, 3 P M Dec 17 1930 3, 11 30 A M Dec 17 1930 4, 11 30 A M Jan 7 1931 5, 11 A M Feb 19 1931 6, 10 A M March 11 1931

The above differences in fragility are evidently produced by some changes in protoplasm but also in the medium surrounding the red cells. How markedly the outer conditions may influence the fragility is seen from Table III where the results of the experiments are given in which men were submitted to a general insolation or to an increase of the temperature of body brought about by an electric current.

In case a curve of hemolysis is wanted four concentrations represent a minimum for the drawing of a curve. For clinical work, however, only the fragility, that is an average of degrees of hemolysis is necessary. In this case the concentration 0.70 per cent influences only little the result and can therefore be omitted.

COMMENT

The method of the determination of the fragility of red blood cells presented in this paper is the only method which yields a correct estimation of the resistance of red blood cells to hypotony using two to five drops of blood. In spite of its simplicity this method is more

exact than former methods and its results do not depend upon the volume of the solutions nor the size of the containers used as is the case in the method of the determination of the threshold of hemolysis. Like Arrhenius' method, the method presented in this paper gives a complete curve of the degree of hemolysis depending upon various concentrations of a salt, or the average fragility of red corpuscles. Some results presented show that only the whole curve of fragility or the average fragility give us a correct idea about the changes of the fragility of red blood cells, because the determination of hemolysis produced by one concentration of a salt solution only, and therefore that of the threshold of hemolysis, give us just the fragility of one kind of corpuscles. For instance in the case of the determination of the threshold only, the fragility of the least resistant corpuscles is tested, while the corpuscles with the greatest resistance or of medial properties react often quite differently. It is therefore not surprising that sometimes the threshold of hemolysis is the same in two specimens of blood while the average fragility of their red cells differ from each other by 100 per cent. Sometimes the threshold of hemolysis may be found at a greater concentration, while the average fragility decreases.

The method presented in this paper has the following advantages in comparison with Arrhenius' method: (1) It requires much less blood (10 to 50 times less). (2) The corpuscles are not centrifuged before they are exposed to the action of hypotonic solutions, every centrifugalization decreases the resistance of red cells to hypotony as it was shown by the author's former experiments (1 c). (3) It does not require a washing and keeping of red cells in salt solutions which change their resistance. (4) In this method not the volume of red cells is considered in estimating the percentage of hemolysis, but their hemoglobin content, since the color of solutions with a partial hemolysis is compared with the color of a completely hemolyzed solution of the same volume of the same blood. This makes the method independent of any changes in the hemoglobin content of red cell which may change for instance by the change of their water content. (5) The red cells are not separated from blood, every separation from blood requires either a shaking with beads which decreases the resistance of red cells (1 c) or a citration which submit the cells to abnormal conditions and change their resistance to hypotony. (6) As the red cells are not separated from blood, not only the changes of fragility produced by some changes in their protoplasm but those produced by some changes in the media surrounding them, are considered in the method presented.

In two experiments taken from the author's not yet published paper it was shown that outer conditions (sun light, high temperature) change the fragility of red cells very distinctly. Red cells are generally considered as real cells the protoplasm of which has the same physico-chemical properties as that of colorless cells. It is therefore very probable that the fragility of colorless cells of our organism is changed similarly.

In the fragility test commonly used in clinical work, only the threshold of hemolysis is determined. Since there can be no doubt that the threshold value is not representative of the total or average fragility of red cells, it might be expected that the clinical use of the method presented here, will possibly change some of the current concepts on pathologic alterations of erythrocytes and it may show that alterations occur under certain physiologic or pathologic conditions in which they have not been known to occur.

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AN IMPROVED METHOD FOR THE DETERMINATION OF BLOOD UREA NITROGEN BY DIRECT NESSLERIZATION*

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INTRODUCTION

THE almost universal recognition of the fact by clinicians that the diminution of blood urea clearance gives evidence of diminishing kidney function earlier than either the phenolsulphonphthalein excretion test¹ or the Mosenthal specific gravity fixation test,² leaves the modern hospital laboratory swamped with a gradually increasing number of requests for urine urea and blood urea nitrogen determinations. Of the blood constituents determined today, none has greater importance than urea nitrogen as one of the factors necessary in determining blood urea clearance as a clinical measure of kidney dysfunction. Of the hosts of methods proposed in recent years, the fact remains that the determination of blood urea nitrogen is still one of the most troublesome procedures encountered in the hospital laboratory. A contributing factor to the lack of uniformity in blood chemical methods is the failure of workers to realize that certain methods are better adaptable than others to multiple determinations as may be required in clinical laboratories in office and hospital while other more complicated methods giving a higher degree of accuracy may be better adaptable to scientific research. In so far as the clinical application of the data obtained is concerned 'undetermined nitrogen' and amino acid nitrogen, most remarkable for its constancy in all of the pathologic conditions thus far investigated would hardly justify distilling off the ammonia from urease-treated tungstic acid blood filtrates before nesslerization in blood urea nitrogen determinations.

From the viewpoint of speed in emergency cases, ease and practicability of manipulation, direct nesslerization would be the method of choice for determining urea nitrogen in blood filtrates if the formation of turbid solutions could be prevented. Several such methods have been proposed, among them the method of Kall,³ and its modifications by Roe and Irish,⁴ Looney⁵ and others, the original method of Kall and its various modifications making use of the tungstic acid blood filtrate of Folin and Wu⁶ in almost all cases. As has been pointed out before by Roe and Irish, Looney, and others, the difficulty encountered in Kall's method is the formation of turbid solutions after the addition of Nessler's reagent, making accurate colorimetric comparison with the nitrogen standard impossible in most cases. To overcome this difficulty many clarifying procedures have been proposed.

Proceeding on the assumption that the turbidity obtained on the direct nesslerization of urease-treated tungstic acid blood filtrates was due to the "precipitation of mercury in Nessler's reagent by traces of proteins and other organic

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matter, in both the urease solution and the substrate," Roe and Irish attempt to solve the turbidity problem by calcium phosphate adsorption of the turbidity producing substances. An extra step is introduced in centrifugation and decantation of the material before nesslerization. In our hands, and in the hands of other workers (Looney, et al.), this method is only partly successful in preventing turbidity.

Approaching the turbidity problem in Kari's method from another angle, Looney saw new possibilities in Folin's use of gum ghatti as a protective colloid to prevent precipitation of Prussian blue in his (Folin's) micro sugar method, and employs this gum as a stabilizing colloid to prevent precipitation of the complex ammonium mercuric iodide compound in Nessler's reagent by the turbidity producing substances present in urease treated tungstic acid blood filtrates. In Looney's method the use of an additional substance (gum ghatti) as a protective colloid in blood filtrates adds to complications because the standard nitrogen solution must contain the same concentration of this substance as the blood filtrate, otherwise the decrease in intensity of color on nesslerization is quite apparent about 10 per cent as pointed out by Looney. Furthermore, the same concentration of the alcoholic solution of urease must be added to the nitrogen standard as to the blood filtrate to guarantee an equal decrease in intensity of color due to this substance on nesslerization. Instead of the aeration method as used in routine work giving figures that are too low, as pointed out by Looney, we believe the contrary is true that Looney's modification gives figures that are too high due to a greater decrease in color intensity on nesslerization of the nitrogen standard than on nesslerization of the blood filtrate, caused by the same concentration of gum ghatti and alcoholic urease solution. One could hardly guarantee an equal decrease in color intensity on nesslerization by adding the same substances, even in equal amounts, to two so widely differing solutions as a simple nitrogen standard and tungstic acid blood filtrates of varying complexity.

The failure of direct nesslerization methods in urease-treated tungstic acid blood filtrates point to the conclusion that before ammonia produced from urea by urease can be nesslerized it must first be separated from interfering urease proteins. We believe that we have been successful in accomplishing this separation by the use of whole oxalated blood and a specially purified and highly concentrated urease reagent, which is completely removed with the total proteins of the blood, according to the Folin-Wu technique of precipitation with tungstic acid (formed by the interaction of sodium tungstate and sulphuric acid) and filtration.

We were especially encouraged to undertake such studies by the researches of Sumner,⁸⁻¹¹ whose work suggested the application of a specially purified and highly concentrated urease reagent as a possible solution of the turbidity problem.

PREPARATION OF THE SPECIAL UREASE REAGENT

Shake 75 gm. Arico (defatted) jack bean meal with 400 cc. of a 32 per cent acetone solution at 28° C. for five minutes. Filter through a fluted filter paper in the refrigerator. Filtration is rapid and the filtrate should come through clear. Add 15 cc. of N/10 acetic acid to the acetone filtrate and allow to remain on ice overnight. Centrifuge and pour off mother liquor. Wash pre-

precipitated urease twice by stirring up with ice cold 32 per cent acetone-phosphate solution, made by diluting c p acetone to 32 per cent with a phosphate mixture containing 143 cc of 0.1 M NaOH and 500 cc of 0.2 M KH_2PO_4 , and centrifuging. (In place of the acetone-phosphate solution, one may wash sediment with ice cold 32 per cent acetone containing 5 per cent N/10 acetic acid, with a reasonably good yield). As pointed out by Sumner, the iso electric point of urease is somewhere in the neighborhood of P_H 6.1, and the precipitated urease is least soluble in the acetone-phosphate solution mentioned above. Pour off the acetone phosphate mixture and drain tubes against filter paper until free from the odor of acetone. Tubes may be placed in the incubator at 37.5°C for a few minutes to free from acetone, if care is exercised not to let the precipitated urease dry, which rapidly inactivates it. Unlike the crude urease preparations, it cannot be dried on paper without complete inactivation. Suspend the washed precipitate of urease in 5 cc of ammonia-free distilled water. Add 0.1 cc of toluene containing 5 per cent thymol as a preservative and keep tightly stoppered on ice in a small bottle. The preparation will keep on ice for at least a month, and in our hands such preparations have developed only a negligible amount of ammonia. The washed sediment will contain the urease in a highly concentrated form (globulin?), and should not give a test for ammonia nitrogen or free carbohydrate.

One drop of the concentrated urease preparation is used for each 5 cc of whole oxalated blood to be analyzed, and represents a urea hydrolyzing power of 178 times the 30 per cent alcoholic 2.5 per cent jack bean extract used in the method of Folm and Svedberg,¹ when made from the same jack bean meal and dropped from pipettes delivering 22 drops per cubic centimeter. One drop of the preparation produced 75.3 mg of urea nitrogen from 25 cc of a 3 per cent urea solution, buffered with 5.4 per cent of K_2HPO_4 and 4.25 per cent of KH_2PO_4 in fifteen minutes at 50°C .

DETERMINATION OF BLOOD UREA NITROGEN

Transfer one volume (5 to 10 cc) of oxalated blood to a flask which has been rinsed in nitric acid and distilled water to free it from mercury. Add one drop of the special urease reagent to each 5 cc volume of blood taken for analysis, and two volumes of ammonia-free distilled water. Stopper tightly and incubate in a water-bath for fifteen minutes at 50°C . Remove from water-bath and add five volumes of water. Add one volume of 10 per cent sodium tungstate solution and mix. Add, with constant shaking, one volume of two thirds normal sulphuric acid. Stopper flask and allow to stand for five minutes. According to the Folm and Wu method of preparing a protein-free blood filtrate, if the color of the coagulum does not gradually change from bright red to dark brown, 10 per cent sulphuric acid must be added from a pipette one drop at a time, shaking after each drop, and continuing until there is practically no foaming and until the dark brown color is apparent. The amount of 10 per cent sulphuric acid that one finds it necessary to add, provided too much oxalate was not used as an anticoagulant, depends on the amount of urea present in the blood which has been hydrolyzed to ammonium carbonate by the urease, and rarely exceeds 4 or 5 drops even in blood high in urea nitrogen.

Pour the mixture on a dry filter paper. The filtrate should be perfectly clear and represents blood diluted 1:10. Place 5 c.c. of the filtrate in a Pyrex tube graduated at 25 c.c., or a 25 c.c. volumetric flask, add 15 c.c. of water, and nesslerize with 25 c.c. of Nessler's solution. Dilute to a volume of 25 c.c. No trace of turbidity should occur on nesslerization. The nitrogen standard for comparison is prepared at the same time by placing 4 c.c. of standard ammonium sulphate solution* (containing 0.4 mg. N) in 100 c.c. volumetric flask. Dilute to about 75 c.c., add 10 c.c. of Nessler's solution, and dilute to the mark. Compare in a colorimeter.

Calculation

$$\frac{\text{Reading of Standard}}{\text{Reading of Unknown}} \times 0.4 \times \frac{100}{0.5} \times \frac{25}{100} = \text{mg. urea N per 100 c.c. blood}$$

When 5 c.c. of the blood filtrate is used, the formula, on solving, becomes

$$\frac{\text{Reading of Standard}}{\text{Reading of Unknown}} \times 20 = \text{mg. urea N per 100 c.c. blood}$$

If the blood filtrate contains an unusual amount of urea nitrogen, of course it is necessary to repeat the nesslerization procedure, using less of the filtrate, in order to obtain a color comparable with the nitrogen standard.

The blood filtrate contains all of the constituents of the blood determined by the Folin-Wu system, without the objectionable presence of urease proteins or other interfering organic matter, and the same filtrate may be used in determining nonprotein nitrogen, uric acid, creatinine, glucose, and chlorides.

EXPERIMENTAL

Two typical experiments will now be cited to show that when the specially concentrated urease preparation is added to whole oxalated blood, it is completely

TABLE I

SHOWING THAT WITH THE IMPROVED METHOD OF DIRECT NESSLERIZATION OF FILTRATE FROM UREASE TREATED WHOLE BLOOD ONE OBTAINS APPROXIMATELY THE SAME BLOOD UREA NITROGEN VALUES AS IN THE AERATION METHOD

Results Are Expressed as mg. Urea Nitrogen per 100 c.c. Blood

BLOOD NO.	UREASE USED DROPS	DIRECT METHOD	AERATION METHOD
1	1	10.4	10.2
2	1	11.0	11.0
3	1	13.0	12.9
4	1	30.2	29.6
5	1	23.0	24.0
6	1	11.8	11.4
7	1	43.7	45.1
8	1	100.0	98.2
9	1	125.5	122.9
10	1	156.3	152.8
11	1	123.0	122.0
12	1	134.3	132.6
13	1	152.0	149.3
14	1	202	198.8

In all determinations the concentrated urease suspension was dropped from a pipette delivering 22 drops per cubic centimeter.

*The standard nitrogen solution is prepared by dissolving 0.4716 gm. of purified ammonium sulphate in a liter of ammonia-free water and is the same standard nitrogen solution commonly used in nonprotein nitrogen determinations.

removed by precipitation with tungstic acid along with the total proteins of the blood

1 Thirty-five cubic centimeters of ammonia-free distilled water was added to 5 c.c. of the concentrated urease suspension, followed by 5 c.c. of 10 per cent sodium tungstate and 5 c.c. of two-thirds normal sulphuric acid. The precipitate which formed was filtered off and the total nitrogen present in the filtrate was determined by the micro Kjeldahl method, employing direct nesslerization. Five cubic centimeters of the filtrate after the micro-Kjeldahl procedure gave no color on nesslerization.

2 Five cubic centimeters of the concentrated urease suspension and 5 c.c. of whole blood containing 25 mg. of nonprotein nitrogen per 100 c.c. of blood were mixed. Thirty cubic centimeters of ammonia-free distilled water was added, followed by 5 c.c. of 10 per cent sodium tungstate and 5 c.c. of two-thirds normal sulphuric acid. The precipitate which formed was filtered off and the non-protein nitrogen again determined on the filtrate, which still remained 25 mg. per 100 c.c. of blood. It is worth while to recall here that only one drop of the enzyme preparation is used for each 5 c.c. of blood analyzed which is sufficient to hydrolyze more than the amount of urea present in 5 c.c. of whole blood within upper pathologic limits.

TABLE II

SHOWING RECOVERY OF ADDED UREA BY THE IMPROVED METHOD OF DIRECT NESSLERIZATION
Results expressed as mg. urea nitrogen per 100 c.c. of blood

BLOOD UREA \	UREA ADDED	TOTAL UREA PRESENT	UREA FOUND	UREA RECOVERED
mg. per 100 c.c.	mg. N	mg. N	mg. N	per cent
22.5	18.6	41.1	41.6	101.1
22.5	74.7	97.2	96.8	99.5
22.5	93.4	115.9	115.3	99.4
22.5	119.8	142.3	143.8	101

TABLE III

COMPARISON OF NONPROTEIN NITROGEN IN BLOOD WITH THE SAME BLOOD TO WHICH ONE DROP OF SPECIAL UREASE REAGENT WAS ADDED AND INCUBATED FOR FIFTEEN MINUTES AT 50° C.

Results expressed as mg. of nonprotein nitrogen per 100 c.c. of blood

SAMPLE NO.	FOUL WU FILTRATE	FOUL WU FILTRATE FROM UREASE TREATED WHOLE BLOOD
1	30	29.8
2	32	33
3	35	35
4	33	33
5	51	51
6	156	153

TABLE IV

SHOWING THAT THE GLUCOSE CONTENT OF WHOLE BLOOD IS NOT AFFECTED BY THE SPECIAL UREASE REAGENT ON INCUBATION FOR FIFTEEN MINUTES AT 50° C

Results are expressed as mg of Glucose per 100 cc of Blood

SAMPLE NO	FOLIN WU FILTRATE	FOLIN WU FILTRATE FROM UREASE TREATED BLOOD
1	74	74
2	68	68
3	194	194
4	191	188
5	195	195
6	98	96
7	100	97
8	84	85
9	99	102
10	300	297

TABLE V

SHOWING THAT THE SPECIAL UREASE REAGENT HAS NO EFFECT ON BLOOD GLUCOSE ON STANDING FOR TWELVE HOURS AT ROOM TEMPERATURE, OR ON INCUBATION FOR FIFTEEN MINUTES AT 50° C

Results expressed as mg glucose per 100 cc of blood

SAMPLE NO (SAME BLOOD)	UREASE USED	ALLOWED TO STAND AT ROOM TEM PERATURE	INCUBATED AT 50° C	GLUCOSE
5 cc	drops	hours	minutes	mg
1	0	0	0	97
2	0	0	15	96
3	1	0	15	96
4	0	12	0	82
5	1	12	0	83

TABLE VI

SHOWING THAT RESULTS FOR URIC ACID AND CREATININE ARE NOT INFLUENCED BY THE CONCENTRATED UREASE REAGENT ON STANDING FOR TWELVE HOURS AT ROOM TEMPERATURE, OR ON INCUBATION FOR FIFTEEN MINUTES AT 50° C

Results expressed as mg per 100 cc of blood

SAMPLE NO (SAME BLOOD)	UREASE USED	ROOM TEMPERA TURE	INCUBATED AT 50° C	URIC ACID	CREATININE
5 cc	drops	hours	minutes	mg	mg
1	0	0	0	34	15
2	0	12	0	34	16
3	0	0	15	34	15
4	1	12	0	33	16
5	1	0	15	34	15

That such a blood filtrate is sufficiently free of uraease proteins, and other interfering organic matter, to justify its use in any of the determinations formerly made on the tungstic acid blood filtrate from blood to which no uraease was previously added is indicated by Tables III to VI

TABLE VII

SHOWING THAT THE TOTAL BUFFERING EFFECT OF WHOLE BLOOD IS SUFFICIENT TO ALLOW THE CONCENTRATED UREASE TO COMPLETELY HYDROLYZE THE AMOUNT OF UREA PRESENT WITHIN HIGH PATHOLOGIC LIMITS, WHEN INCUBATED FOR FIFTEEN MINUTES AT 50° C AND THAT THE ADDITION OF ANY SPECIAL BUFFER SOLUTION IS UNNECESSARY

Results in mg urea nitrogen per 100 cc of blood

BLOOD UREA N	UREA ADDED	TOTAL UREA PRESENT	UREA FOUND	UREASE USED	PHOSPHATE BUFFER USED*
mg per 100 cc	mg N	mg N	mg N		
17.1	186.9	204	206.0	1 drop	none
17.1	186.9	204	202.2	2 drops	none
17.1	186.9	204	200.1	4 drops	none
17.1	186.9	204	201.6	0.5 cc	none
17.1	186.9	204	200.8	0.5 cc	0.5 cc
17.1	186.9	204	204.2	0.5 cc	1.0 cc
17.1	186.9	204	197.9	0.5 cc	1.5 cc
17.1	186.9	204	205.3	0.5 cc	2.0 cc

*The phosphate buffer used consisted of 6.9 gm of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ and 17.9 gm of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ dissolved in 100 cc of ammonia-free water. All tests were made on 5 cc samples of the same blood.

SUMMARY

1 A new method for the determination of urea nitrogen in blood has been developed. The urea in whole oxalated blood is hydrolyzed to ammonium carbonate by the action of a specially purified and highly concentrated uraease reagent in fifteen minutes at 50° C. The concentrated uraease (globulin?) is completely removed with the total proteins of the blood by precipitation with tungstic acid, in the preparation of the Fohn-Wu protein-free blood filtrate. Urea nitrogen is determined colorimetrically, without turbidity interference, after nesslerization of the blood filtrate.

2 The use of a protective colloid to prevent turbidity due to precipitation of the complex ammonium mercuric iodide in Nessler's reagent by uraease proteins in blood filtrates is obviated.

3 The method requires no more blood than is required by other methods, and compares favorably in speed and accuracy with any existing method.

4 The method offers a degree of ease and practicability of manipulation which allows many determinations to be made at the same time, requires no additional apparatus, and eliminates technical difficulties and inaccuracies of distillation procedures.

5 The total buffering effect of whole oxalated blood is sufficient to maintain

a reaction suitable to allow one drop of the concentrated urease reagent to completely hydrolyze the amount of urea present in 5 c.c. of blood, even within upper pathologic limits, without the addition of any special buffer solution to the blood.

6 The blood filtrate contains all of the other constituents of the blood determined by the Folin-Wu system, unchanged, and the same blood filtrate may be used in such determinations.

7 The purified and highly concentrated reagent requires a minimum amount of time for preparation, particularly in consideration of its keeping qualities on ice, when preserved with 0.1 c.c. of toluene containing 5 per cent thymol for each 5 c.c. of the reagent.

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SUPRAVITAL DIFFERENTIAL COUNTING ADAPTED TO CLINICAL USE*

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THE supravital method for differential counting has not been generally adopted for clinical use because of two drawbacks in the technic usually employed. The first of these is that the differential must be made *shortly after preparing the blood film*, since it is a common experience that after the lapse of about two hours the cells have taken up large amounts of the dye and cannot be identified. The second is the requirement of a specially constructed warm chamber which is expensive and often is not available. A method has been developed which eliminates these difficulties. This method is presented here, together with comparative results obtained by its use.

METHOD

The method proposed differs from that usually employed^{1, 2} in three respects. First, in the use of a more dilute solution of the dye; second, the preservation of the blood film in the ice box until the count can be made; and third, the substitution of a simple lighting system for the warm chamber. The stock solution of dye is made up in the usual way, 125 mg. of neutral red iodide No. 2 dissolved in

*From the Laboratories of The Rockefeller Institute for Medical Research.

50 c c of absolute alcohol. The slides are coated with a dye consisting of 30 drops of the stock solution in 10 c c of absolute alcohol instead of the usual 100 drops.* When the blood film has been prepared and sealed with vaseline as by the ordinary method, the count may be made at once, or the preparation may be stored in the ice box at a temperature of about 4°C until ready for the examination. In making the counts a warm chamber is unnecessary. As the source of illumination, an ordinary 40 watt frosted electric light bulb suspended on an iron stand has been found inexpensive and satisfactory.

Through the use of this procedure, a preparation which has been kept in the ice box for twenty-four hours appears to be entirely similar to a fresh smear and cannot be distinguished from it, all cells have been found to be actively motile and to retain their morphologic, viable characteristics. Smears left in the open laboratory at ordinary room temperature (20°C) were found to last about four hours or twice as long as those kept at 37° in the warm chamber. Poor results in delayed counts by this method are usually due either to improperly cleaned slides and cover slips or to thick smears.

COMPARATIVE RESULTS

The accuracy of delayed differential counts is evidenced by our experience summarized in Tables I and II. In a preliminary report² parallel observations on six male rabbits were recorded. A series of 16 smears was counted immediately after taking the blood, with the usual technique, and after twenty-four hours in the ice box, a duplicate set of smears was counted with the modifications here described. In making this comparison, 5300 cells were counted on a total of 32 smears. The means and standard errors of the means of the two series of counts are given in Table I.

TABLE I
RESULTS OF IMMEDIATE AND DELAYED SUPRAVITAL COUNTS FIRST SERIES

Age of Smear in Hours	NEUTROPHILS		BASOPHILS		EOSINOPHILS		LYMPHOCYTES		MONOCYTES	
	0 2	18 24	0 2	18 24	0 2	18 24	0 2	18 24	0 2	18 24
Means	59.0	57.6	3.6	4.7	0.9	0.8	23.4	24.7	12.5	12.0
Standard Error of the Mean	± 2.1	± 1.4	± 0.4	± 0.6	± 0.2	± 0.2	± 2.2	± 1.9	± 1.1	± 1.4

That comparable results were obtained is seen from the fact that no significant difference between the respective means was obtained, and in no case was this difference equal to twice its standard error.

Further experience with this method has confirmed our original observations. Duplicate sets of smears were made on 14 normal male rabbits, 3 rabbits infected with *Treponema pallidum*, 3 normal men, and 3 men with active syphilis, 46 smears in all. A total of 3400 cells was counted immediately on one set of

*The following is the method of preparing the slides and blood films as described by Sabin. The surface of a clean slide is flooded with the diluted stock dye solution. The excess of dye is drained off the slide which is then allowed to dry. Slides stained in this manner keep indefinitely. Blood films are made by placing a small drop of blood on a clean cover slip which is then inverted gently on the slide. The cover slip is rimmed with vaseline and the blood films are now ready for study.

smears, following the usual technic, and 3400 cells were counted on the duplicate set after preservation in the ice box for twenty-four hours. The results are shown in Table II.

TABLE II
RESULTS OF IMMEDIATE AND DELAYED SUPRAVITAL COUNTS SECOND SERIES

Age of Smear in Hours	NEUTROPHILES		BASOPHILES		EOSINOPHILES		LYMPHOCYTES		MONOCYTES	
	0 2	18 24	0 2	18 24	0 2	18 24	0 2	18 24	0 2	18 24
Means	53.2	54.1	4.1	4.6	2.3	2.2	30.4	29.6	9.8	9.3
Standard Error of the Mean	± 1.9	± 1.6	± 0.6	± 0.8	± 0.6	± 0.5	± 1.9	± 1.6	± 0.8	± 0.9

Here again, there is no significant difference between the respective means, and this difference in no case equals twice its standard error.

The method has also been employed in isolated examinations of blood from sources other than those shown in the tables. Excellent results have attended delayed differentials made on rabbits inoculated with a malignant tumor, on rabbits with severe snuffles, on men with various anemias, myelogenous leucemia, infectious mononucleosis, and syphilis.

SUMMARY

A modification of the supravital technic for differential counting is here proposed which makes available to the clinician and general practitioner a method heretofore largely limited to scientific laboratories.

It renders possible accurate differential white blood cell counts with the supravital technic as long as twenty-four hours after making the preparation. This is accomplished (1) by reducing the concentration of the dye used, (2) by placing the blood smears in the refrigerator until ready for counting, and (3) by substituting a simple lighting arrangement for the warm chamber.

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NEW HARTMAN TRANSFUSION APPARATUS*

F W HARTMAN, M D , DETROIT, MICH

A NEW apparatus for citrate transfusion has been evolved which embodies the advantages of citration at the needle described in previous publications¹ and increases the simplicity, smoothness, and safety of the procedure

DESCRIPTION OF APPARATUS

A one liter Pyrex bottle (*B*) with a connection at the bottom for the blood tube (*A*) is used. This blood tube consists of a No 22 catheter. There is a needle connection at the end as illustrated. This connection and that with the bottle are tied to withstand the pressure used in giving the blood. The top assembly (*D*) is the regular aspirator bottle type. It has the usual two way connection with the bottle and valves which open and close the same. Small additional handles are placed on these valves to facilitate smooth operation. Attached to the core of the top assembly (*D*) a glass dropper and tube (*C*) are attached with a heavy rubber connection covering the end of the core but leaving the side opening through which suction or pressure may be produced in the bottle by attaching a two way aspirating pump to tube and connection (*F*). On the lower end of the dropper and tube (*C*) a small No 8 catheter is attached which is cut long enough to run through the blood tube (*A*) to the connection at the end. Now it is seen that, with the connection and tube (*L*) which is long enough to reach the bottom of the citrate flask (*H*), a complete citrate system is formed so that when negative pressure is produced in the bottle, citrate is drawn from flask (*H*) through (*E*) and (*C*) to the end of the small catheter where it is released in the blood tube (*A*) and in turn reaches the bottle (*B*). If blood is coming from the vein through (*A*) at this time the citrate is mixed with the blood as it leaves the needle and flows to bottle (*B*).

The top assembly is held in place by a clamp (*K*) and screwed into position by a set screw (*G*). The end of this set screw (*G*) is attached to the top assembly (*D*), so that during sterilization the top assembly may be held suspended, thus avoiding molding of the rubber stopper. The citrate flask (*H*) is held by a spring-steel clamp which may be detached. Attached to this clamp is a scale showing the volume of fluid in the bottle. The citrate flask is graduated and holds 120 cc.

OPERATION OF APPARATUS FOR TRANSFUSION

The apparatus is carefully washed and rinsed with distilled water. Then, with the bottle (*K*) half full of water, the small catheter is allowed to flow into the blood tube with the water. The entire apparatus, except the pump and needles, is wrapped and steam sterilized at 15 pounds for twenty minutes. Care must be taken not to bend tubes sharply or sterilize at higher temperatures. At

*From Department of Laboratories, Henry Ford Hospital
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the time of use the top assembly (*D*) is screwed down into place with (*G*). The citrate flask (*H*) is filled with 2 per cent citrate solution. A two way aspirating pump is attached to (*F*) and negative pressure within the bottle, sufficient to draw citrate through the apparatus and leaving 15 c.c. in the bottle (*B*), is produced. If necessary the gloved finger or a sterile sponge may be held over the end of (*A*) during this procedure. The arm of the donor is now prepared and a blood pressure cuff placed above the elbow. The cuff is distended above diastolic

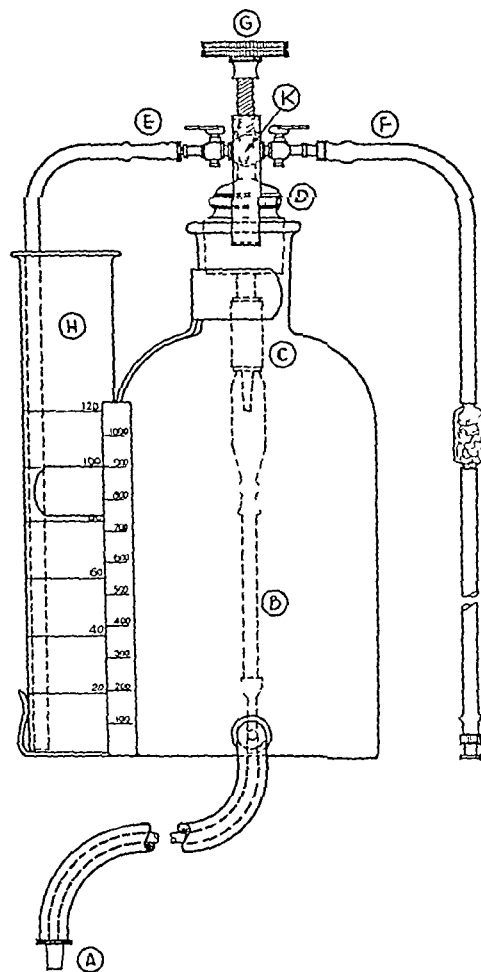


FIG. 1

pressure and the needle plunged cleanly into the best available vein. When a good flow of blood is obtained tube (*A*) is attached to the needle. More negative pressure is now produced within the bottle (*B*) to obtain a good flow of blood and also to bring the citrate over from (*H*) through (*C*). The flow of citrate is observed as it comes through the dropper and must be kept flowing constantly, but the amount may be regulated by the valve shown near (*F*), so that there will be approximately 12 to 15 c.c. of the 2 per cent citrate to each 85 c.c. of blood drawn. The bottle may be gently rotated but never shaken.

When the desired amount of blood is obtained the pump is disconnected and the negative pressure released. The pressure in the blood pressure cuff is released and the blood tube (A) compressed between the fingers and the needle withdrawn from the donor's vein. The tube (A) is held high for drainage, and placed in clip in side of top assembly clamp (H) to prevent the loss of blood from the bottle (B).

The patient's arm is prepared and a needle placed in the vein. The blood tube (A) is lowered until it is filled with blood, then the connection is made with the needle. The aspirating pump is reversed, and, after closing the citrate system with the valve near (E), positive pressure is produced in the bottle (B) sufficient to effect a steady flow of blood into the patient's vein.

SUMMARY

A new apparatus for citrate transfusion is presented which has the advantages of citration at the needle thus preventing pre-coagulative changes but which avoids the use of the special double shouldered needle thus allowing the selection of any needle according to the vein of the donor. The dropper is placed within the bottle and the top assembly is such that there is no difficulty in holding the stopper in place even when considerable pressure is exerted within the bottle. The citrate is regulated by the small valve near (E) instead of the screw clamp. (This apparatus has been modeled for me by Mr. Cox of J. F. Hartz Company 1529 Broadway, Detroit, Michigan, and may be obtained from them.)

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A SIMPLE VACUUM-TUBE POTENTIOMETER FOR THE MEASUREMENT OF GLASS ELECTRODE POTENTIALS*

H. B. VAN DYKE, PH. D., M. D., AND RALPH D. BINNETT, PH. D., CHICAGO, ILL.

IN THE measurement of the potentials of the glass electrode it is necessary to employ a method which not only is sensitive but also draws no appreciable current from this high-resistance electrode. The advantages of the method given in this paper lie in the simplicity of the circuit and the ease of operation. These advantages depend upon the employment of the General Electric Company's "Photron" FP 54. It was later discovered that Hill¹ used this same tube for a similar purpose. However, the circuit which we employ is somewhat simpler; moreover, the only shield required, enclosing the tube, control-grid switch, and glass-electrode lead to the control-grid switch, forms a single compact unit.

The control-grid of this vacuum tube is very highly insulated and is stated by the makers to have a leakage-resistance of about 10^{16} ohms. This character-

*From the Pharmacological Laboratory and the Department of Physics, University of Chicago, Chicago.
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istic, of course, is of the greatest importance and enables one to make measurements at the "free" grid potential without further complication of the circuit since any negative potential in this tube is essentially a "free" grid potential. The current drawn by the control-grid is of the order of 10^{-10} amperes. Besides the filament, the tube also contains a space charge grid. The filament, space-charge grid, and plate can all be operated from a single 6-volt storage battery since there is a potential of only 6 volts on the plate. The mutual conductance of the tube is 25 microamperes per volt.

A diagram of the circuit is shown in Fig. 1 in which a Leeds and Northrup type K potentiometer is employed. The control-grid switch (SW_1) is described in detail below. Switch 2 (SW_2) enables one to reverse the current of the potentiometer cell. Switch 3 (SW_3) is required when the galvanometer is used for the standardization of the potentiometer current. B_1 is a lead storage battery, B_2 is a 1.5 volt dry cell (a Burgess "C" battery is satisfactory). B_3 , B_4 ,

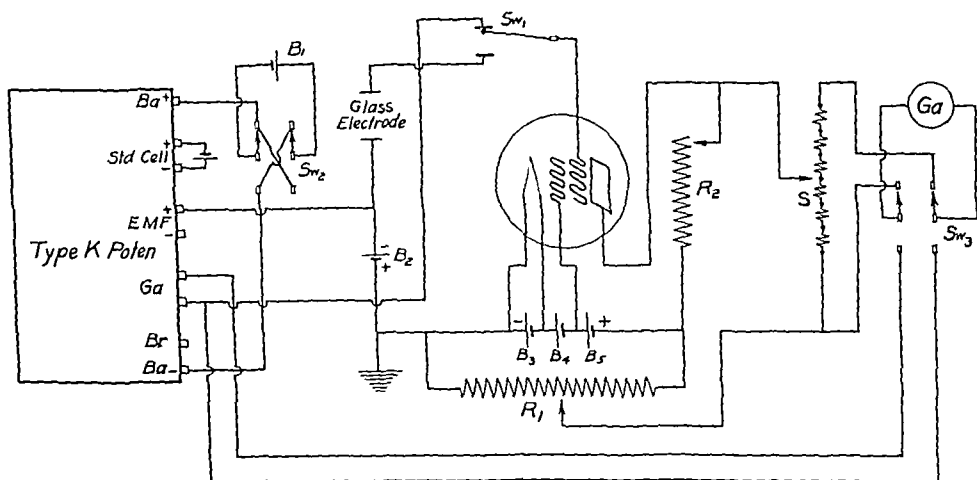


Fig. 1

and B_5 are the three cells of an automobile storage battery. The potential divider R_1 should have a total resistance of several thousand ohms to avoid undue drain on the battery. It should be wire-wound. R_2 is a resistance of about 10,000 ohms which may be kept fixed. S is an Ayrton shunt of a resistance (3,000 ohms) appropriate for the galvanometer used. The galvanometer is a Leeds and Northrup type R instrument with a sensitivity of 0.003 microampere per millimeter.

The tube and control grid switch (SW_1) must be completely shielded. A satisfactory arrangement is shown in Fig. 2. All dimensions are given in inches. The parts labelled A, B, and C, are made of brass and are grounded through a binding post soldered in A. After a careful cleansing with alcohol and ether, the tube is kept dry by means of P_2O_5 . A phosphor-bronze strip is soldered to the control grid lead at the top of the tube. Into the free end of this strip is soldered a platinum pin which makes contact with E and F through platinum. Contact through F is made by depressing the quartz-rod plunger D. The binding post of F may be used for connecting it with the silver wire of the silver-silver chloride

half-cell of a glass electrode of the type devised by MacInnes and Dole² if the electrode is inserted in the electrode-thermoregulator of Stadie, O'Brien and Lang³. On the other hand, if one wishes the glass electrode to be held in a vertical position, a side arm consisting of two pieces of brass tubing soldered together at a right angle and containing a copper wire imbedded in sulphur may be slipped over C. One end of the copper wire is fastened in the binding post of F, the other end (in the vertical limb of the tube) is clipped to the silver wire of a glass electrode which is immersed in the solution, the P_H of which is being determined. Wires are soldered to the four prongs in the base of the tube for connecting the filament terminals, the space charge grid, and the plate to the storage

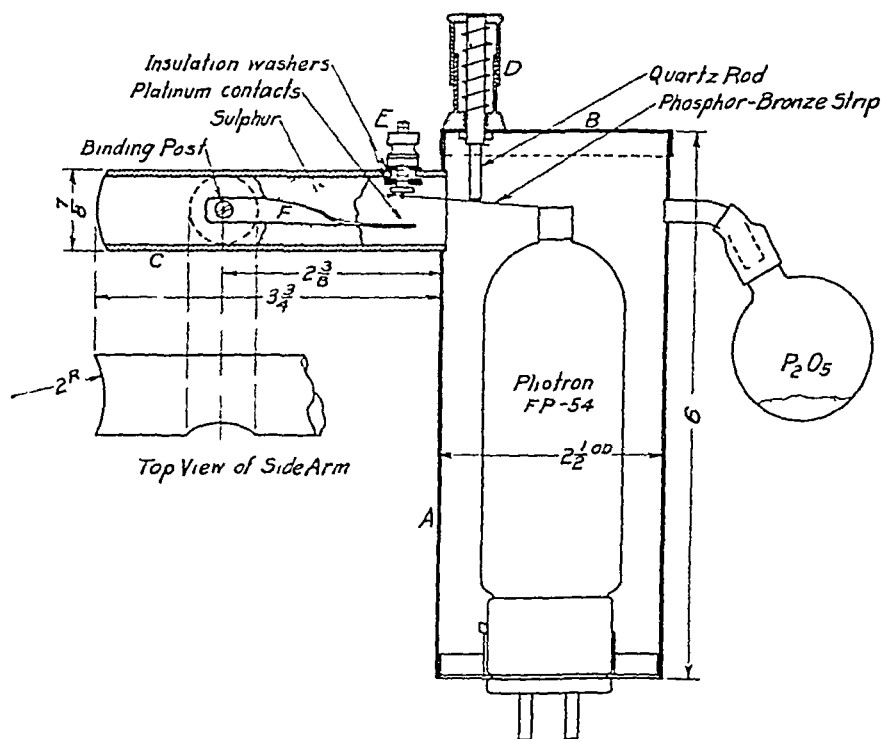


Fig. 2

battery, these connections may remain permanently and need not be interrupted while the battery is being charged.

OPERATION OF THE POTENTIOMETER

The principle of the method employed is to apply alternately to the control-grid a known fraction of the potential of the potentiometer-cell B₁, and the unknown potential of the glass electrode. When these two potentials are identical the grid potential will remain constant when the plunger is depressed. Consequently, the plate current of the tube will also remain constant. The object of the branch circuit R₂, R₁, S, and G_a is to determine when such constancy exists.

The action of the circuit is briefly as follows: the plate current through R₂ produces a potential drop across it of a few tenths of a volt, so that the actual

plate potential is a few tenths less than the total of batteries B_1 , B_1 , B_1 . The slider on R_1 is then adjusted to give this same potential. If now the galvanometer is connected between the slider and the plate, it will show no deflection. Further if SW_1 is now depressed and the choice of potentiometer voltage was correctly made so that the potentials of E and F (Fig 2) are identical, the galvanometer still will not deflect. Under these conditions the potential of the glass electrode, being the same as that of E (Fig 2), can be read directly on the potentiometer. The purpose of the Ayton shunt is to reduce the sensitivity of the galvanometer during preliminary adjustments.

The adjustment need not be made so that the galvanometer deflection is actually zero with the Ayton shunt set for maximum sensitivity. It must, however, be sufficiently near zero to keep the image on the scale. The important requirement is that the image does not move as SW_1 is depressed.

TABLE I
COMPARISON OF DETERMINATION OF P_H OF PHOSPHATE BUFFERS BY HYDROGEN AND GLASS ELECTRODES

NO	P_H BY HYDROGEN ELECTRODE*	P_H BY GLASS ELECTRODE
1	6.441	6.430
2	7.067	7.055
3	7.275	7.270
4	7.478	7.466
5	7.664	7.659
6	7.860	7.841

*Determined by Dr. M. E. Hanke

Under conditions of maximum sensitivity for the shunt a change of potential of 1 millivolt causes a deflection of the galvanometer image of 4.4 to 4.7 mm. If the potential of the control-grid bias (B_2) is increased to -3 volts, the sensitivity is reduced to 2.9 to 3.1 mm per millivolt. This is because at the greater grid bias the tube operates on a less steep part of the grid potential-plate current characteristic. The drift of the galvanometer image is not sufficient to interfere with estimates of the accuracy indicated in the examples given below. While the maximum drift which has been observed is 0.3 mm per minute, ordinarily it is about 0.2 mm per minute.

In Table I are given examples of determinations of the P_H of buffer solutions by the hydrogen electrode and by the glass electrode. The glass electrode determinations were made at room temperature and undoubtedly could be improved by more adequate temperature control. Some idea of the reproducibility of determinations is furnished by eight successive determinations on a phosphate-buffer of P_H 7.51, the P_H values found were 7.505, 7.508, 7.513, 7.512, 7.502, 7.497, 7.497, and 7.509. These also were determined at room temperature.

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A CLASS EXPERIMENT ON CO-ENZYMIC ACTIVITY*

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THE armamentarium of reliable laboratory experiments which can be used by senior students in the study of enzymic processes to demonstrate for themselves the specific effect of an activator on an enzymic reaction, is somewhat limited.

The following experiment, which takes advantage of the very striking effect of Mg ions on the phosphatase activity of rats' (or dogs') red blood cells¹ has been recently devised for such students and may be carried out without difficulty in any laboratory possessing a colorimeter.

MATERIAL REQUIRED

(a) Six cubic centimeters of rats' or dogs' red cells (beet or human red cells give a much smaller activation) from oxalated or defibrinated blood. These are washed once with 0.85 per cent NaCl solution, and laked by adding 18 c.c. of distilled water containing chloroform, and if necessary by freezing and thawing. After keeping for one hour the laked or almost completely laked preparation is centrifuged, and the upper layer removed from the stromata, calcium oxalate and unlaked cells.

(b) One hundred fifty cubic centimeters of a 0.30 per cent solution of sodium β -glycerophosphate (or the commercial glycerophosphate will serve), adjusted to P_H 7.4.

(c) Eighty-five one hundredths per cent sodium chloride solution.

(d) A few cubic centimeters of M/5 (approx.) magnesium chloride solution, and the same quantity of M/5 (approx.) calcium chloride solution, both adjusted to P_H 7.4.

(e) Twenty-five per cent trichloroacetic acid solution.

METHOD

In Table I are given directions for setting up the experiments. Ordinary test tubes fitted with rubber stoppers are used.

Since in the absence of added enzyme no hydrolysis of sodium glycerophosphate takes place in twenty-four hours under these conditions, controls containing glycerophosphate (with and without $MgCl_2$ or $CaCl_2$) but no red cell extract have been omitted from Table I, but may be set up at the same time if thought desirable.

PROCEDURE

To four tubes A, A₁, B, and B₁, 2 c.c. of 25 per cent trichloroacetic acid are added immediately. After mixing and standing for not more than fifteen minutes, the contents of these four tubes are filtered and the filtrates, carefully stoppered, are kept in the refrigerator. Filtration without undue delay is necessary to avoid errors which may arise from the hydrolytic action of the trichloroacetic acid, even in the cold, on the lipins of the red cells. All the rest of the tubes are

*From Department of Biochemistry, University of Toronto.
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TABLE I
DIRECTIONS FOR SETTING UP A CLASS EXPERIMENT TO DEMONSTRATE MAGNESIUM ACTIVATION OF PHOSPHATASE

NO OF TUBES	TUBE LABELS	0.3% NA β GLYCERO PHOSPHATE	WATER	0.9% NaCl	M/5 MgCl ₂	M/5 CaCl ₂	DILUTED RED CELLS	CHCl ₃ DROPS	TYPICAL RESULTS Mg P	SHOWING
2	A, A ₁	10	-	2	-	-	1	3	0.022	{Hydrolysis in absence of Mg
2	B, B ₁	10	-	2	-	-	1	3	0.074	
2	C, C ₁	10	-	1.8	0.2	-	1	3	0.304	{Marked activation by approx 0.003 M Mg
2	D, D ₁	10	-	1.8	-	0.2	1	3	0.063	
2	E, E ₁	-	10	2	-	-	1	3	0.021	{Slight inhibition by approx 0.003 M Ca
2	F, F ₁	-	10	2	-	-	1	3	0.053	
2	G, G ₁	-	10	1.8	0.2	-	1	3	0.056	{Autolysis controls
2	H, H ₁	-	10	1.8	-	0.2	1	3	0.055	

incubated in a water thermostat at 38° C for from twelve to twenty-four hours, with occasional mixing. At the end of this time the tubes are cooled to room temperature, 2 c.c. of trichloroacetic acid are added to each tube, and the contents mixed. After standing fifteen minutes the contents of these tubes are filtered through phosphate-free filter papers, and the inorganic phosphate is determined on aliquots (10 c.c. or less) of the filtrates and of the filtrates from A, A₁, E, and E₁, using any convenient micro-method for inorganic P determination (e.g. Briggs, Bell-Davis, Fiske-Subbarow). It is advisable to separate the aliquots into duplicate series of eight filtrates each, and to use 0.1 and 0.3 mg P as standards for 10 c.c. aliquots. For convenience in reading, 0.05 mg P is added to all the flasks containing aliquots except C before adding the colorimetric reagents, and is then subtracted after the reading has been made.

Typical inorganic P contents of 10 c.c. aliquots are shown in Table I. In this particular experiment rats' red cells were used. An activation of several hundreds per cent is shown.

FURTHER EXPERIMENTS

Since most of the autolyzable phosphoric esters of the red cells are hydrolyzed under the experimental conditions just described, it is not shown by this experiment whether or not the presence of MgCl₂ activates the autolysis ("Phosphatolysis") process. This point can be taken up with the senior students if desired. The effect of other cations related to Mg may be examined also, and it will be found that the activation effect is, apparently, quite specific for Mg.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M D, ABSTRACT EDITOR

HEPATIC FUNCTION Van den Bergh Reaction and Bromsulphalein Test in the Estimation of, Cantarow, A. *Am J M Sc* 181 215, 1932

Cantarow compares these two methods in the study of hepatic function in 188 patients and concludes that the analysis of observations on the degree of bilirubinemia, bromsulphalein retention and the van den Bergh reaction in a group of 188 patients showing abnormal response to one or more of these studies appears to indicate that the type of dye reaction is not determined solely by the serum bilirubin content.

The type of van den Bergh reaction appears to bear a more direct relation to the degree of bromsulphalein retention than to the degree of bilirubinemia except in cases of extra hepatic obstructive jaundice.

There is no direct correlation between the type of van den Bergh reaction and the plasma cholesterol concentration.

Repeated determinations of the van den Bergh reaction and the observation of its transition from one type to another in individuals with biliary tract disease are of considerable significance, if interpreted in conjunction with the estimation of the degree of bilirubinemia and bromsulphalein retention.

Of 234 patients with cholecystitis with and without cholelithiasis, 70 presented either hyperbilirubinemia or bromsulphalein retention or both. Of these 34 were cases of chronic cholecystitis, without cholelithiasis and with no other demonstrable evidence of hepatic disease.

In 3 cases of gall bladder disease there was 100 per cent retention of bromsulphalein at the end of 30 minutes, associated with icterus index values of 8.3, 7.2 and 8.7. These findings suggest dissociation of these two phases of the excretory function of the liver.

This disturbance of hepatic function in patients with gall bladder disease appears to be largely "functional" in nature and associated with but slight demonstrable organic disease of the liver parenchyma.

More careful routine preoperative study of patients with surgical disorders of the biliary passages and proper preoperative treatment will perhaps greatly diminish the post operative morbidity and mortality of biliary tract surgery.

LIPEMIA Variations in the Total Blood Lipid Wechsler, H F. *Arch Int Med* 50 37, 1932

The total blood lipid curves after the ingestion of 100 cc of olive oil in sixty seven subjects who were apparently free from pancreatic or hepatic disease could be subdivided into three categories: ascending, 63.2 per cent, flat, 17.5 per cent, and descending, 19.3 per cent.

The age of the subject and the presence of arteriosclerosis markedly influenced the total blood lipid. Persons in the second and third decades presented a flat curve, those in the fourth, fifth and sixth decades an ascending curve and those showing evidence of arteriosclerosis a flat or descending curve.

Starvation produced a moderate progressive rise in the total blood lipid.
Dextrose eliminated the starvation effect.

CULTURE MEDIUM From Soy Bean, Kostriko, D S, and Maryash, T K. *Lab Pratika*, Moscow 8 10, 1932

The following is described as an inexpensive medium efficacious for the growth of most pathogenic bacteria, especially suitable for anaerobes, and for bacterial counts.

Three methods of preparation are described.

1 A soy bean extract is prepared by adding 100 gm of soy beans to 1 liter of water, boiling from thirty minutes to one hour, filtering, and sterilizing by autoclaving twenty minutes at 120° C

2 Several soy beans (eight or ten) are added to a test tube with water and sterilized like the extract

3 Soy bean agar is prepared by adding from 15 to 2 per cent agar to the extract and sterilizing

BLOOD SEDIMENTATION TEST in the Management of the Pneumothorax Patient, Cutler, J W Am Rev Tub 26 134, 1932

The sedimentation test was studied in a group of 131 patients receiving artificial pneumothorax treatment during the past three and a half years. The purpose in view was to ascertain what help, if any, the physician may expect from the test in the management of such cases. The following observations and deductions were made:

1 The sedimentation test fills a gap in the management of tuberculous patients receiving artificial pneumothorax treatment. It gives valuable information at a time when x-ray findings are obscured and physical signs and symptoms obliterated by the collapse.

2 The sedimentation rate is a sensitive measure of the activity of the tuberculous process in the compressed lung and is the last objective evidence of activity to become normal. A return to normal indicates quiescence of the pathologic process but does not necessarily indicate stability of the lesion.

3 In uneventful cases the sedimentation rate shows steady improvement and changes from a vertical or a diagonal curve, indicating activity of the tuberculous process, to a horizontal line, indicating quiescence, but not until weeks or months after the disappearance of constitutional signs and symptoms.

4 When there is disease in both lungs and the more involved lung has responded to pneumothorax treatment, the sedimentation rate is a simple means to determine the effect of the compression on the less involved side. It will not become normal until the tuberculous process is quiescent in both lungs.

5 During the early part of the treatment the sedimentation test should be repeated at least once a month and afterward every two months. A diagonal line with an index of 15 mm or more should always be respected. The patient should be warned that the disease is active and that a relapse is possible. Exercise should be prescribed with great caution and its effect studied carefully by means of the sedimentation test regardless of how well the patient may feel clinically. Adherence to this rule will prevent many a relapse and spread in the opposite lung.

6 The test is simple to perform and to interpret. By its proper utilization the physician can gather valuable information out of all proportion to the effort made to obtain it. Further study should make this one of the most widely used clinical laboratory procedures in phthisis therapy.

TUBERCLE BACILLI Rapid Staining of, in Sputum, Doglio, P Giorn di Batt e Immun Turin, 8 243, 1932

Applying the principle, first proposed by Spengler, that red acid fast organisms are readily detected against a yellow background, Doglio proposes the following contrast stain:

Brilliant yellow	0.15 gram
Sulphuric acid conc	10 c c
Alcohol	20 c c
Distilled water	85 c c

This is used after carbol fuchsin in the usual manner, as both a decolorizing solution and control stain. The bacilli are red on a lemon yellow background.

GONORRHEA Unreliability of Laboratory Aids in the Diagnosis of, in Women, Jacoby A. Am J Obst & Gynec 23 729, 1932

The following conclusions are advanced:

1 Repeated smears should be taken and carefully examined.

2 The use of Gram's stain is not essential. In conjunction with a proper evaluation of the clinical examination, the methylene blue stain is adequate for practical purposes.

3 A positive smear is conclusive evidence of infection.

4 A negative smear, even when repeated, does not exclude the presence of a gonococcal infection in women.

5 Suspicious organisms, extra or intracellular, should be interpreted in accordance with the clinical evidence.

6 Pure spreads of pus cells, even without organisms present, should be regarded as suspicious evidence of gonococcal infection.

7 When cultures are taken and prove positive, they constitute criteria, but are not practical or well adapted to routine practice.

8 A negative culture does not exclude the presence of a gonococcal infection.

9 The complement fixation test for gonorrhea with the present technique is unreliable. Neither positive nor negative findings are conclusive.

10 Unless an improved technique affording more reliable results is evolved, the fixation test should not be used for the diagnosis of gonorrhea or for the control of its treatment.

Even under the most favorable conditions it is apparent that laboratory procedures are of minor importance in establishing a diagnosis of gonorrhea in women. A wider appreciation of this fact with a consequently greater reliance upon the history and clinical evidence, will suggest the correct diagnosis in many of the now unrecognized cases of gonorrhea in women.

TUBERCLE BACILLI A Simplified Egg Medium for the Cultivation of, Feldman, W. H. *Am Rev Tub* 26 187, 1932

PREPARATION OF MEDIUM

Seven fresh eggs are washed in warm water and immersed for ten minutes in 80 per cent alcohol.

A portion of the shell is carefully broken at one end of the eggs, and the membranous sac is punctured with sterile, sharp pointed scissors. The egg white is discarded and the yolks are discharged into a sterile mixing bowl.

To the egg yolks are added 100 cc of a 6 per cent solution of glycerin prepared as follows: glycerin 24 cc, distilled water 300 cc sterilized in the autoclave for fifteen minutes at 15 pounds' pressure. The solution can be autoclaved in 100 cc portions and stored for future use.

The egg yolks and the solution of glycerin are thoroughly mixed with a sterile egg beater and tubed, using sterile apparatus. Precautions are taken to minimize possible contamination.

The sterilizing is done in the Arnold sterilizer or the inspissator, on the first day at 75° C until solidified, then at 85° C for one hour, and on the second, third and fourth days at 75° C for one hour. Before the medium is used it should be incubated for two days at 37° C. It is best stored in the refrigerator, and drying can be minimized by placing the tubes in large cylindrical museum jars, smearing the contact surfaces of the jar and the lid with petrolatum. A few crystals of thymol seem to assist materially in controlling molds.

SEDIMENTATION TEST A Comparison of the Sedimentation Test and Ruge Virulence Test in 150 Gynecologic Cases, Simunich, W. A. *Am J Obst & Gynec* 23 724, 1932

In the Ruge virulence test 5 cc of blood drawn from the cubital vein under aseptic precautions is defibrinated by shaking for five minutes in a sterile glass tube containing glass beads. One half cc of this blood is placed in each of two tubes one of which is inoculated with 2 or 3 loopfuls of vaginal or cervical secretion and the other is kept as a control. Smears are made from the contents of both tubes which are immediately there after incubated at 37.5° C. Smears are repeated at hourly intervals for three hours and stained with methylene blue. If the organisms increase within three hours the test is positive, i. e., organisms pathogenic for the host are present in the genital canal.

The following conclusions are drawn from a study of 150 cases.

1 An increase in sedimentation speed of 60 minutes or less was observed in more than 50 per cent of the cases of inflammatory adnexitis, uncomplicated and complicated fibroids and the carcinomata, and in about 23 per cent of other abdominal and gynecological pathology not of an inflammatory nature

2 The presence of virulent organisms is one of the most important causes of postoperative morbidity and mortality but a doubtful or positive virulence test does not depend on the speed of sedimentation

3 The increase in sedimentation speed is due to some other factor than the virulence of organisms

4 The sedimentation test is not a reliable guide in the determination of the time for safe operation of adnexal disease

5 The Ruge virulence test is of value in the prognostication of postoperative morbidity and mortality if the operation takes place at the site of the organisms, usually the cervix

6 The history, white and differential counts, temperature and physical examination must remain our main guides in the determination of the time for safe operation in adnexal disease, while in cervical and combined cervicoadnexal operations the Ruge virulence test is of undoubted value. A doubtful or positive virulence test contraindicates cervical operations until such a time that the test becomes negative

BLOOD Blood Picture in Sickle Cell Anemia, Diggs, L. W. Southern M. J. 25 615, 1932

The blood elements in sickle cell anemia, although subject to wide variations, present during the definitely anemic phases of the disease a diagnostic blood picture. Sickled cells in the fixed smear are rarely specific enough to make the diagnosis on morphology alone. The suspicion is usually aroused by the history and clinical signs, by an anemia associated with jaundice, leucocytosis and premature erythrocytes in the smear. Given such a combination in a negro, the demonstration of the sickle cell phenomenon in the moist preparation clinches the diagnosis. Reticulocyte count, cell volume, fragility test and examination of the family for sickling would be confirmatory procedures indicated. It is predicted that sickle cell anemia will become a common diagnosis when clinicians become sickle cell anemia minded and in addition to personally studying blood smears, make moist preparations routine in unexplained hemolytic anemias.

The characteristic features of the blood picture in active sickle cell anemia are

(a) The presence of sickled cells in moist preparations

(b) Signs of red blood cell destruction (poikilocytosis, degenerating forms, nucleocytes, increased serum bilirubin, negative direct van den Bergh and positive indirect, urobilinuria, phagocytosis of erythrocytes by large mononuclears)

(c) Signs of increased regenerative activity on the part of the bone marrow (megalo blasts, intermediary forms, nucleated red blood cells, nuclear fragments of all types, diffuse basophilia macrocytes, increased reticulocytes, leucocytosis, with a shift right and left, increased platelets)

Sickling without anemia, hemolytic anemia without sickling, or secondary anemia in association with the sickle cell trait are to be distinguished from true sickle cell anemia.

Typical sickled cells, although striking and unmistakable in fixed smears and fresh moist preparations from some cases, are the exception rather than the rule.

The average red blood cell in sickle cell anemia is smaller than normal. The cell volume is decreased out of proportion to the decrease in the red blood cell count and hemoglobin. The color index is variable but usually below 1.

The erythrocytes in sickle cell anemia are more resistant to hypotonic salt solutions than normal.

SUGAR TOLERANCE, Phosphates In, McCullagh, D. B., and Van Alstine, L. Am. J. Clin. Path. 2 277, 1932

The phosphate changes in the blood of normal individuals after the administration of glucose show considerable regularity.

The changes in blood phosphate after the administration of glucose to patients suffering from metabolic disorders frequently differ from the changes in blood phosphate in normal individuals

It is impossible to make a definite differential diagnosis in various metabolic disorders by means of the phosphate curve

B ACIDOPHILUS, Medium for, Kulp, W L, and White, V Science 76 17, 1932

Mixture A—Add 10 gm Difco peptone and 10 gm Difco peptonized milk to 400 cc of juice filtered from a good quality of canned tomatoes. Heat this mixture gently to dissolve the peptone and peptonized milk. Unnecessary heating of the tomato juice should be avoided. The reaction of the solution is changed to P_H 6.0 to 6.2. There should be little deviation from this suggested reaction.

Mixture B—Add 11 gm dried agar to 600 cc distilled water and autoclave this mixture to dissolve the agar.

Just previous to the removal of Mixture B from the autoclave, bring Mixture A to the boiling point. Then mix A and B while both are hot and filter through a thin layer of absorbent cotton. Distribute the filtered medium in containers (test tubes preferred) and sterilize by heating in the autoclave at $120^{\circ}C$ for eight minutes.

LEISHMANIA A New Solid Medium for the Cultivation of L. donovani, Salle, A. J, Brit J Infect Dis 49 475, 1932

Peptone (Difco) 20 gm, beef infusion 250 cc, salt 5 gm, agar 15 gm and distilled water to make 830 cc are boiled to dissolve the agar. Water is added to replace loss by evaporation, and the reaction is adjusted to P_H 7.2. The agar base is distributed into flasks (800 cc to each flask) and sterilized in the autoclave. When required, to one flask is added 200 cc of sterile 50 per cent dextrose solution and the agar is melted. When cooled to $50^{\circ}C$ 15 cc of defibrinated rabbit blood is added and the mixture distributed in test tubes to solidify as slopes. On the surface of this medium in five or six days at $22^{\circ}C$ massive quantities of organisms are obtained. The liquid medium is composed of peptone 20 gm, beef infusion 250 cc, salt 5 gm, dextrose 10 gm and distilled water to make 550 cc. The reaction is adjusted to P_H 7.2 with sodium hydroxide. To this mixture is added a mixture of 500 cc of distilled water and 150 cc of defibrinated rabbit's blood which has been allowed to stand till complete hemolysis has taken place. The resulting 1000 cc of medium is centrifuged till clear and sterilized by filtration through Seitz or Berkefeld filters. The medium should be used in a shallow layer at the bottom of a flask.

HEMOPHILIA Mechanism of, in Infancy and Childhood, Kugelmass, I N Am J Dis Child 44 50, 1932

The following conclusions are presented:

1 The criteria necessary and sufficient for the diagnosis of hemophilia have been formulated on the basis of experimental and clinical studies.

2 Quantitative determinations of the constituents involved in blood coagulation show the hemophilic deficiency to be in the primary stage of the blood clotting mechanism.

3 Hemophilic blood shows a strikingly low prothrombin content compensated by a correspondingly high antithrombin content. The platelets are normal in number but are physiologically defective having a slow rate of disintegration.

4 Hemophilic blood is characterized by a blood clotting index that is less than one-tenth of the normal. The index constitutes the ratio of the concentration of the substances tending to clot over those tending to favor bleeding. Accordingly, the normal index of clotting function is 0.5, whereas the hemophilic clotting index is less than 0.05. This very low index of blood clotting function is diagnostic of hemophilia.

5 The index of blood clotting function shows a manifold increase after transfusion but the improved clotting lasts for only forty-eight hours. Transfusion does not alter the clotting function of blood in hemophilic subjects with manifestations in the joints.

6 Dietary protein, lipids, vitamins or minerals do not alter the deficient clotting function in hemophili.

7 Hemophilic persons show the absence of the female sex hormone in their tissues normally present in males. Ovarian therapy, theelin and other products of the female generative organs injected into the hemophilic subject produce no change in the clotting mechanism evaluated quantitatively.

8 Serum injected or applied locally is not effective in controlling hemorrhage in hemophilia, unless it is fresh and rich in thrombin. But nonhemophilic bleeders respond readily to any serum and to dietary protein therapy.

SUGAR TOLERANCE Clinical Evaluation of Blood Phosphate and Sugar Tolerance Curves, Hartman, F. W., and Foster, D. P. *Am J Clin Path* 2: 289, 1932

Five hundred combined glucose tolerance and phosphate curves were taken on patients considered potential diabetics. One hundred were from individuals 25 to 100 pounds overweight. These show an increased rise of the blood glucose with slow fall while the inorganic phosphates decreased moderately with slow recovery.

One hundred two combined glucose tolerance and phosphate curves from individuals normal in weight or undernourished, showed high elevation of the glucose curve with gradual return to the fasting level while the phosphate curve showed only slight depression with slight recovery.

Twenty five combined glucose tolerance and phosphate curves on mild or moderate diabetics showed typical diminished glucose tolerance curves. The phosphate curves showed slight and continued depression.

The curve of inorganic phosphates is a valuable supplement to the glucose tolerance curve in the diagnosis of abnormal carbohydrate metabolism.

LEUCOCYTES White Blood Cell Counts in Convalescence From Infectious Diseases, Reznikoff, P. *Am J M Sc* 184: 167, 1932

While admitting the usefulness and value of the hemograms emphasis is laid upon the fact that it is only one nonspecific factor and that its real value arises from sequential counts at frequent intervals rather than isolated determinations.

What a patient shows at a particular moment is only of importance insofar as it throws light on the direction a patient is going.

In recovery from an acute infectious disease three more or less distinct phases may be demarcated by the hemogram: subsidence of the acute stage, convalescence, complete return to normal.

The subsidence of the acute stage is characterized by a decrease in immature polymorphonuclears and a marked, even if transitory, rise in monocytes.

Convalescence is characterized by a normal immature polymorphonuclear count, a marked rise in lymphocytes (often above 40 per cent) and a variable eosinophilia.

Complete recovery is characterized by a return of the lymphocytes to a normal range.

MUSEUM SPECIMENS, Mounting of, Caylor, H. D. *Science* 75: 517, 1932

Discarded X-ray film may be used for mounting of small specimens of light weight.

The emulsion is removed by soaking in hot water and scraping. The dried film is then cut to the desired size to make an exact fit for the inside of the museum jar, and the fixed specimen sewed in place. Fixing fluid is then added and the jar sealed. The advantages of the method are: (1) suspension of specimen on an invisible material, (2) film transparent, allowing view of both sides, (3) film is waste material, (4) the cumbersome glass frame is unnecessary.

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EDITORIAL

Ulcer Vs Carcinoma

IT IS inevitable, and, from the nature of the situation inescapable, that in the consideration of medical matters in general there should be many instances in particular concerning which there is a difference of opinion.

A subject of perennial debate, of which much has been written and still more said, is that of gastric ulcer as concerns the proper, most advisable, and most satisfactory method of treatment. On this subject physicians and surgeons have carried on for years a perpetual debate of which the end is not yet in sight, for each has experience and statistics to support his contention, vigorously combated by the differing experience and statistical data of his opponent.

While it is relatively easy to outline generalities applicable to abstract concepts, it is not always easy to apply them successfully to particular instances, and so the discussion of the medical versus the surgical treatment of gastric ulcer continues with temporary victory resting now with one side and now with the other.

It cannot be disputed, of course, that there are cases in which the results of medical treatment, carefully planned and faithfully followed, have been eminently satisfactory from the clinical standpoint and in which no disagreeable aftermath has been recorded.

It is equally true that there are other cases which, despite the seemingly satisfactory results, eventually have reached the surgeon.

On the other hand, it is also true that the surgical treatment of ulcers has had its failures as evidenced by the recurring ulcers and the patients who have not found gastroenterostomy and its like a panacea.

It is not strange that this should be the case and that statistical data should show such wide and marked differences, for it is beyond question that many "cures" attributable to medical treatment, and an equal number following surgical intervention are "cures" only because no consistent effort is made to assure a thorough "follow-up" of the case, or because the patient, for one reason or another, passes out of observation, many later to appear in some other set of records.

In the last analysis gastric ulcer is not in itself a disease but the manifestation of an underlying mechanism, the precise nature of which remains to be demonstrated.

As always, it is not the disease which must be treated but the patient who has it, and as long as the conditions which lead to ulcer production continue to exist in a particular patient, no treatment can be considered as final in its results.

The surgeon who confronts the internist with the fact that his experience has failed to demonstrate complete epithelialization following medical treatment, may be confronted in turn by the case in which, despite a beautiful and efficient short circuiting, recurring and severe hemorrhage dominate the later history.

The ulcer patients stand between the Scylla of hemorrhage on the one hand and the Charbydis of malignant transformation on the other and much of the complaint of the surgeon rests upon the fact that all too often the ulcer has become a carcinoma by the time it reaches his hands.

It is certainly true that there are many gastric ulcers which, for various reasons, economic or otherwise, may well be subjected to medical treatment. There are others, however, in which the beginning of malignant changes renders immediate operative interference imperative.

Obviously, then, the evolution of methods whereby the imminence of malignant transformation may be foretold would be of the highest practical significance.

The studies of Alvarez and MacCarty¹ on the relation of the size of gastric ulcers to the development of malignant changes are of great as well as practical interest.

This study was conducted to establish, if possible, the contention of MacCarty, Carman, and others of the Mayo Clinic that any chronic *gastric* ulcer with a crater more than 2.5 cm. in diameter (as established roentgenographically), is probably the seat of cancerous change and should be subjected to surgical rather than medical treatment.

It is quite apparent that, if this contention be true, a criterion is available

whereby malignancy arising from gastric ulcers may be properly treated while it is still in the operable stage

From the percentage distribution curves based on areas of 638 resected gastric ulcers and 682 gastric carcinomas resected during this study, it appears that four out of five benign ulcers are less than 1.8 cm. in diameter (smaller than a dime), and ninety-two out of the one hundred are less than 2.4 cm. in diameter (smaller than a quarter)

Of the carcinomas resected in the series, 23 per cent fell within the range of size of benign ulcers so that, on the basis of size alone, there is one chance in ten that an ulcer smaller than a quarter is already cancerous, two to one that, if larger than a quarter but smaller than a silver dollar, it is a cancer, while it is almost certainly cancerous if larger than a dollar

These studies indicate the essential importance of roentgenologic examination of all patients with persistent gastric symptomatology and surgical intervention in all who present ulcers with a crater more than 2.5 cm. in diameter, appreciating, of course, that even then cases will be missed because of inability to demonstrate the lesion with sufficient clearness to enable its accurate measurement

REFERENCE

1. Alvarez, W. C., and McCarty, W. C. Size of Resected Gastric Ulcers and Gastric Carcinomas, *J. A. M. A.* 91: 226, 1928

—R. A. K.

ITEM

The American Society of Clinical Pathologists

The following men were made honorary members of the American Society of Clinical Pathologists

Charles Achard Professor of Clinical Medicine at the Faculty of Medicine in Paris, member of the Institute and the Academy of Medicine

Dr. F. Craig, Col. U. S. Army, Retd., Director of Department of Tropical Medicine, Tulane University, New Orleans, La.

Dr. E. C. Dodds, M.V.C., M.D., Ph.D., B.S., B.Sc., M.R.C.P., Courtauld Professor of Biochemistry in the University of London, Chemical Pathologist to the Middlesex Hospital

INDEX TO VOLUME XVII

AUTHORS INDEX

In this index following the author's name the title of the subject is given as it appeared in the JOURNAL. Editorials are also included in the list and are indicated by (E)

A

AHLFELDT FLORENCE D (See Custer and Ahlfeldt) 960

B

BARNARD ROBERT D A cyanhematin standard for the Sahli hemoglobinometer 824

BARROW WILLIAM H The acid response of the stomach to test meals of protein, fat and carbohydrate 1094

BAUMAN L (See Pickens and Bauman) 820

BEECROFT RUTH (See d'Herelle and Beecroft) 667

BENNETT RALPH D (See van Dyke and Bennett) 1268

BLACKMAN NATHAN (See Kopeloff and Blackman) 85

BLAIR, WILLIAM M (See Taylor and Blair) 1256

BOSCH, WALTER C (See Williams and Bosch) 196

BOWER ALBERT G (See Meals and Bower) 409

BRAINARD D H (See Noble and Brainard) 573

BRICE ARTHUR T JR The clinical incidence of tryptophanuria 65

C

CASEY ALBERT D AND ROSAHN, PAUL D Supravital differential counting adapted to clinical use 1263

CAVETT, J W An improved micro Kjeldahl method 79

COBE HERBERT MARSHALL Gingivitis chemotherapy as an aid in the diagnosis and treatment 437

COLEMAN MARION B (See Gilbert and Coleman) 88

CONNERY JOSEPH E AND GOLDWATER J II Studies on patients with pernicious anemia treated with massive doses of liver extract Effects on reticulocytes red cells hemoglobin and white cells 1016

COOK, JEROME E (See Sievers and Cook) 1120

CORKE DOREEN R (See Tolstoi and Corke) 450

COWIE D MURRAY AND HICKS WM C Observations on the Bacteriophage III The treatment of colon bacillus infections of the urinary tract by means of subcutaneous and intravascular injections of bacteriophage filtrates Detailed case reports Methods for preparation of filtrates 681

CROWLEY CHARLES F A simple method for the analysis of protein in milk 373

CUSTER R P Studies on the structure and function of bone marrow I Variability of the hemopoietic pattern and consideration of method for examination 951

— AND AHLFELDT FLORENCE F Studies on the structure and function of bone marrow II Variations in cellularity in various bones with advancing years of life and their relative response to stimuli 960

D

DACEY, H GLADYS (See Gilbert and Dacey) 345

DEWITT, CHARLES B An improved distilling column 199

DIGGS, L W The sickle cell phenomenon I The rate of sickling in most preparations 913

DILLMAN L M The effect of ultraviolet irradiation on glucose solution 236

— The effects of ultraviolet irradiation on the reducing power of blood, 44

DOAN, CHARLES A Current views on the origin and maturation of the cells of the blood 887

DUNLAP J H (See McCullagh and Dunlap) 1060

E

EAGLE HARRY Studies in the serology of syphilis VII On the supposed artificial induction of a positive Wassermann reaction in originally negative human sera 778

— Studies in the serology of syphilis VIII A new flocculation test for the serum diagnosis of syphilis 787

EARLE WALTER C AND PEREZ MANUEL Enumeration of parasites in the blood of malarial patients 1124

EDMUNDS, CHARLES W AND SMITH RALPH G Experimental adrenal exhaustion 399

EILMANN H J (See Ramsey and Eilmann) 415

EIMAN, ROBERT, AND TAUSSIG, J B The quantitative determination of cholesterol in the bile 274

ELTON NORMAN W Physiology correlations and technic of the van den Bergh reaction icterus index and quantitative serum bilirubin I

— Relation of jaundice to the outcome in lobar pneumonia indications for the trial of bilirubin therapy, 216

EWERT EARL E (See Herrold and Ewert) 49

F

FAMULENER L W Preparation of vaccines 538

FANZ JOHN I AND GAULT, EDWIN S The Aschheim Zondek test modified, for diagnosis of early pregnancy 354

FABER JASON E (See Youngburg and Faber) 363

FAUGHT FRANCIS ASHLEY The significance of the periodic health examination and its influence upon the health of a group of examinees 337

FAUST ERNEST CARROLL Examination and identification of protozoa 639

FELSEY JOSEPH A device used with mounted intestinal specimens to stimulate sigmoidoscopic views 380

FELSHIN GERTRUDE (See Frank Goldberger and Felshin) 61

FITZ-HUGH THOMAS JR The age of the leucocyte in relation to infection 975

FLASHMAN DAVID H A microscopic arrangement for reading macroscopic Kahn precipitation tests 382

- FOSHAY, LIE A new method for staining bacterium tularensis in tissue sections 193
- FOSTER, R. H. K. An improved ether bottle for animal anesthesia 490
- FRANK, FANNIE CHESNUTT (See Havens and Frank) 1155
- FRANK, ROBERT T. GOLDBERGER, MORRIS A. AND FELSHIN GERTRUDE The value and limitations of the Aschheim-Zondek pregnancy test 61
- FRY, WILLIAM L. (See Webster and Fry), 452

G

- GARRISON, ALLEN D. A simplified instrument for measuring metabolism 814
- GALT, LEWIS S. (See Kim and Gault) 354
- GAY, DOUGLAS M. Cooper's modification of the Ziehl-Nelson staining method as applied to tubercle bacilli in tissue 1131
- GIFFIN, HERBERT Z. Diagnostic features of the blood count and of the morphology of the blood in diseases associated with splenomegaly 1050
- GILBERT, RUTH The broader aspects of routine cultural examinations 507
- AND COLEMAN, MARION B. A simple presumptive test for agglutination with organisms of the abortus melitensis group 88
- AND DACH, H. GLADYS The isolation of an organism of the abortus melitensis group from a blood clot the serum of which failed to give agglutination with B. abortus 346
- GOLPNER, ALFRED (See Steel Goerner, and Haley) 139
- GOLDBERGER, MORRIS A. (See Frank Goldberger and Felshin) 61
- GOLDHAMMER, S. M. Special features of the blood in infancy and childhood 1043
- GOLDWATER, LEONARD J. (See Connery and Goldwater) 1016
- GORDON, BURGESS Aneroid type of tambour for recording respiratory movements and intrathoracic pressure 812
- A recording type of artificial pneumothorax apparatus 75
- GORDON, ETHEL M. (See White and Gordon) 53
- GORDON, HAROLD A simple apparatus for cleaning coverslips 190
- GRUSKIN, B. Intradermal test for the determination of malignancy 1237
- GUTHRIE, C. C. An apparatus for quickly measuring the specific gravity of body fluids 1158
- A split second timer 376

H

- HADEN, RUSSELL L. A simple apparatus for the transfusion of blood by the citrate method 1027
- The march of hematology 948 (E)
- The technique of a blood examination 813
- HALEY, FRANK L. (See Steel Goerner and Haley) 19
- HALL, O. G. A laboratory chronograph providing intermittent and constant current from a direct current line of 110 volts 485
- HARTMAN, F. W. New Hartman transfusion apparatus 1266
- HASKINS, HOWARD D. (See Osgood Haskins and Trotman) 859
- HAVENS, LEON C. The isolation and identification of pathogenic bacteria in feces 628
- AND FRANK FANNIE CHESNUTT The Kohn precipitation test the use of a single tube with the optimum proportion of serum and antigen 1155
- D'HERELLE, F. AND BECCIOFT, RUTH Bacterial mutations 667
- HENROLD, RUSSELL D. AND EWERT, EARL F. The relation of the PH reaction of urine to the antiseptic action of mallophone in vitro 49

- HICKS, W. J. C. (See Cowie and Hicks) 681
- HILL, ROBERT M. A permanent nitroprussid solution for acetone tests 375
- HOLT, HELEN (See Moench and Holt), 297

I

- ISAACS, PAPHALL Present status of the study and treatment of leucemia 1006

J

- JAMIESON, W. A. (See Marks Powell and Jamieson) 413
- JENNEL, H. D. AND KAY, H. D. A class experiment on co enzymic activity 1272
- JOHNSON, CARL A. The effect of amyl nitrate upon the finger volume, 59

K

- KAHN, BERNARD S. (See Leiboff and Kahn) 77
- KASHAN, MAXWELL (See Krasnow Karshun and Krejci) 1145
- KAY, H. D. (See Jenner and Kay) 1272
- KELLY, ROBERT A. Vaccines in clinical medicine 515
- KELLER, ALEXANDER G. JR. A micro method for blood urea nitrogen 1146
- KILBUELL, ROBERT A. Experimental studies of dengue 794 (F)
- Malignancy in radioactive persons 840 (F)
- The leucocytic reaction of D. Amato in the study of disease 1180 (E)
- The specific therapy of pneumonia 107 (F)
- Ulcer vs. Carcinoma 1282 (E)
- KIM, M. S. The effect of liver extract on bile pigment formation 1223
- KLING, DAVID H. AND PINKUS, JULIUS The gonococcus complement-fixation test in synovial fluid 9
- KOELLÖFF, NICHOLAS AND BLACKMAN, NATHAN A new Petri dish holder for counting and fishing colonies 85
- KRACKE, ROY P. A review of granulocytopenia (agranulocytosis) 977
- KRAEKA, JOSEPH JR. Endogenous uric acid and hemopoiesis III Uric acid outputs and reticulocyte counts as affected by glycine caffeine urea bilirubin atophan and xylene 428
- KRASNOW, FRANCES KASHAN, MAXWELL AND KREJCI, LAURA F. The determination of calcium and phosphorus in saliva 1148
- KRIDLER, WILLIAM A. AND SMALL, JAMES C. A method of standardizing colloidal gold sols by utilizing a standard solution of globulin 269
- AND — A method for the standardization of colloidal gold sols in the Lange test 259
- KREJCI, LAURA F. (See Krasnow Karshun and Krejci) 1148
- KUENZEL, W. M. (See Sommerfeld Kuenzel and Todd) 151

L

- LAKE, MICHAEL (See Lauss Lake and Torrey) 109
- LARUM, A. W. Bacteriophage in clinical medicine 675
- LAROCHELLE, F. D. Modification of use of Wright's stain 818
- LAWSON, HAMPHREY (See Templeton and Lawson) 1244
- LEDERER, M. (See Polyzes and Lederer) 1029
- LEGERE, HELEN (See Richter Meyer and LeGERE) 1185
- LEIBOFF, S. L. AND KAHN, BERNARD S. A simple clinical procedure for the determination of urea in urine by means of hydrolysis 77
- LEPLSCHAY, W. W. Fragility of red corpuscles and its determination in clinical work 1250
- LEVINE, B. S. A transparent rule for measuring basal metabolism graphs 826

- FINA JAMES R. The decompensated hypertensive heart 211
 LOVE, J. W. Variations in the diameter of the granulocytes 942
 LANCH, KENNETH M. Intestinal protozoa in clinical medicine 661
 LION, B. B. VINCENT. The bacteriology of bile obtained by duodenal tube biliary drainage 553

M

- MCUREA, ADELIA. A proposed standard method for the estimation of fungicides, 72
 MCCULLAGH, D. ROY (See McCullagh and McCullagh), 754
 MCCULLAGH, D. P. AND DUNLAP, J. H. The blood picture in hyperthyroidism and in hypothyroidism 1060
 — AND MCCULLAGH, D. ROY. Carbohydrate in the treatment of postoperative tetany, with special reference to lactose 751
 MCNAMALA, W. L. A rapid paraffin technique 1162
 MADDOCK, STEPHEN J. A new procedure for ligating the pylorus in absorption experiments 369
 MARKS, H. H. POWELL, H. M. AND JAMESON, W. A. Merthiolate as a skin disinfecting agent 443
 MARSDEN, C. S. JR. (See Pickard, Pierce, Marsden, Tanaka and Townsend), 471
 MARTIN, LAY AND MORGENSTERN, MORTON. Carbon dioxide changes in alveolar air and blood plasma or serum after subcutaneous histamine injection in human beings 1228
 MASS, MORRIS AND SCHAFFER, NATHAN. A device designed to simplify the handling of celloidin sections 821
 MEARS, ROBERT W. AND BOWER, ALBERT G. Polomyelitis 409
 MIDLAR, E. M. A critical study of the polynuclear count as advocated by Schilling 169
 MEERER, WILLIAM R. A comparison of the vasoconstrictive action of adrenalin and ephedrine added to the local anesthetic solution 773
 MEYER, ARTHUR E. (See Richter, Meyer and Legere), 1185
 MEYER, K. F. Use of animals in routine diagnostic work 510
 MICKLE, FRIEND LEE (See Welch and Mickle), 67
 MILLER, H. F. The leucocytes in surgical conditions 1100
 MILLS, C. A. Hemophilia 932
 MILTON, P. (See Obermer and Milton), 792
 MOENCH, G. L. AND HOLT, HELEN. Biometrical studies of head lengths of human spermatozoa 297
 MORGENSTERN, MORTON (See Martin and Morgenstern), 1228
 MORRISON, MAURICE. An analysis of the blood picture in 100 cases of malignancy 1070
 MORSE, WITHROW. A simple apparatus for absorbing substances from the expired air of laboratory animals 282
 MYERS, VICTOR C. The use of hydrogen peroxide in the micro Kjeldahl nitrogen method 272

N

- NAUSS, RALPH W. LAKE, MICHAEL AND TORREY, JOHN C. A critical analysis of the Lyon bile drainage technique as an aid to bacteriologic diagnosis 109
 NEEDLES, ROBERT J. A neutrophilic graph 962
 NICHOLLS, EDITH E. (See Stainsby and Nicholls), 530 566
 NOBLE, W. C. JR. AND BRAINARD, D. H. The bacteriology of the nose and throat 573
 NORTON, JOHN F. Bacteriology of pus 558
 NOVI, I. G. Perspiration of microorganisms 731

O

- OBLEMER, L. AND MITTON, R. The Veines Briceq-Yvon photometer. Its application to routine biochemical work with special reference to the estimation of phosphorus in blood, 792
 OSGOOD, EDWIN L. HASKINS, HOWARD D. AND THOMAN, FRANK E. The value of acurately determined color, volume and saturation indexes in anemias, 859

P

- PARR, LEONARD W. The presence and significance of isohemagglutinins in the body outside of the blood stream 333
 PEREZ, MANUEL (See Eyle and Perez), 1124
 PICKARD, RAWSON J. AND RICE, CLARA. Method for staining fecal protozoa 493
 — PIERCE, LEO F. MARSDEN, C. S. JR. TANAKA, R. K. AND TOWNSEND, H. A. A nonglucose reduction present in normal and increased in nephritic blood, 471
 PICKLINS, MARJORIE AND BAUMAN, L. The estimation of bilirubin in blood serum, 820
 PIERCE, L. F. A new and simplified technique of surgical photography, 81
 — (See Pickard, Pierce, Marsden, Tanaka, Townsend), 471
 — AND RICE, CLARA S. Determination of Ph values of urine 1133
 PINKUS, JULIUS (See Kling and Pinkus), 39
 PINNER, MAX. Sputum examination in pulmonary tuberculosis 611
 PLUMMER, NORMAN. Laboratory methods in the treatment of pneumonia, 594
 POLAYES, S. H. AND LEDERER, M. Reactions to blood transfusion. Observations from 2500 transfusions with a review of the literature, 1029
 POWELL, H. M. (See Marks, Powell and Jameson), 443

R

- RALLI, CLAUDE P. Liver extract in the treatment of diabetes mellitus 1204
 RAMSEY, THOMAS L. AND DILMANN, H. J. Carbon monoxide acute and chronic poisoning and experimental studies 415
 REICH, CARL A. Diagnostic aid in obscure hematologic conditions 198
 REID, WILLIAM D. Preliminary report on the use of an improved form of electrocardiograph 304
 REINHART, J. ARTHUR. A new test tube rack for use in serology and bacteriology 485
 RICE, CLARA (See Pickard and Rice), 493
 — (See Pierce and Rice), 1133
 RICH, MURRAY L. (See Speckman and Rich), 165
 RICHTER, OSCAR MEYER, ARTHUR E. AND LEGERE, HELEN. The value of aqueous equine liver extract glycerated iron and hemoglobin in the treatment of secondary anemias 1185
 ROBINSON, WILLIAM. A simple thermocouple and a thermopile for determination of temperature in biology and medicine 181
 ROBINSON, W. L. Mechanical aids in laboratory procedures 251
 ROSAHN, PAUL D. (See Casey and Rosahn), 1263
 ROWE, ALLAN WINTER. Note on the calculation of urine solids 466

S

- SCHAFFER, NATHAN (See Mass and Schaffer), 821
 SCHULTZ, W. H. An operating table for small animals 1153
 SHAW, FREDERICK W. Examination for pathogenic fungi 549

- SIFVERS EDWARD F. AND COOK JEROME I. The competency of the Richfuss tube as a complete evacuator, 1120
- SMALL, JAMES C. (See Kieffler and Small), 259 264
- SMITH PAUL G. (See Edmunds and Smith), 399
- SOMMERFIELD W. A. Studies in the alimentary canal of man. IX. The calculation of gastric volume, 240
- KUENZEL, W. M. AND TODD T. WINGATE. Studies in the alimentary canal of man. VIII. The time relationship of gastric peristalsis, 151
- SOULE M. H. Microbic technic, 519
- The symposium (on clinical bacteriology), 605 (F)
- SPECKMAN, RUSSELL A. AND RICH MURRAY L. An analysis of fifty heart cases showing low voltage, 165
- STABLER N. GRAHAM. A new device for honing microtome knives on glass, 375
- STAINSBY, WENDALL J. AND NICHOLLS, EDITH E. Bacteriologic examination of blood and spinal fluid, 566
- AND — Technic for the isolation of streptococci, 530
- STELL MATTHEW GOLDBERGER ALFRED AND HALPER FRANK I. Biochemical and pharmacologic study of quinine bisulfate salicylate, 139
- STOUT B. F. Septic cavernous sinus thrombosis. Report of two cases with recovery of one following bacteriophage therapy, 28
- STRICKLER ALBERT AND ZALATEL RALPH P. A comparative statistical study of the frequency of asymptomatic ringworm as occurring in the more common cutaneous affections, 748
- STURGIS CYRUS C. Treatment of the anemias, 1010
- SWANSON EDWARD L. The detoxification of cocaine, picrotoxin and strychnine by sodium amygdal, 325
- T
- TANAKA R. K. (See Pickard Pierce Marsden Tanaka and Townsend), 471
- TAUSSIG J. B. (See Elman and Taussig), 274
- TAYLOR WALTER T. AND BLAIR WILLIAM M. An improved method for the determination of blood urea nitrogen by direct nesslerization, 1256
- TEMPLETON R. D. AND LAWSON HAMPDEN. Technical contributions to the study of gastrointestinal motility, 1244
- TODD T. WINGATE. (See Sommerfield Kuenzel and Todd), 151
- TOLSTOI EDWARD AND CORKE DOREEN R. Treatment of rheumatic fever with a magnesium cinchophen magnesium oxide (magnaphen) preparation, 450
- TOMPKINS EDNA H. Clinical applications of supravital staining, 921
- TORREY, JOHN C. (See Nauss Lake and Torrey), 109
- TOWNSEND, H. A. (See Pickard Pierce Marsden Tanaka and Townsend), 471
- TROTMAN FRANK E. (See Osgood Haskins and Trotman), 859
- TSUCHIDA, H. A practical staining method for intestinal protozoa, 1163
- A survey of intestinal protozoa among children in St. Louis, 133
- TYNER, JAMES D. The prediabetic state. Its treatment by the low carbohydrate diet and the reduction of weight, 456
- V
- VAN DYKE, H. B. AND BENNETT, PAUL D. A simple vacuum-tube potentiometer for the measurement of glass electrode potentials, 1268
- VALGREN WARREN T. Protein digestion and food allergy, 503 (E)
- The effect of carbon monoxide poisoning on the heart, 208 (E)
- The treatment of nephritis, 292 (E)
- W
- WADDILL J. A. The action of iverin on voluntary and nonvoluntary muscle, 1104
- WALKER BURNHAM S. Normal relationships of blood and urine phosphorus, 347
- WALTON ROBERT P. The sterilization and standardization of pupal preparations intended for surgical use, 459
- WEBSTER ROBERT K. AND FRY WILLIAM E. A manometer for magnification of blood pressure tracings, 482
- WELCH HENRY, AND MICHAEL, FRIEND LEE. Comparison of the Huddleston slide test with a macroscopic tube test in undulant fever, 67
- WHITE E. P. CORSOV. Study on a series of arthritic patients under continuous mono-iodo-cinchophen treatment with special reference to the action of the cinchophen molecule on the liver tract, 17
- WHITE T. D. AND GORDON ETHEL M. The estimation of the serum crotin, 53
- WIBEL CHARLES L. The catamenia and oxygen consumption, 14
- WILLIAMS JOHN W. Nonelastic bulb for pipettes, 280
- AND BOSCH WALTER C. Nonpolarizable electrodes, 196
- WINFIELD G. A. A study of the white and differential counts in six unselected cases of inoculation malaria, 985
- WINTROBE M. M. The size and hemoglobin content of the erythrocyte, 899
- WOHL MICHAEL G. Diabetes mellitus and the gastric secretion, 22
- Y
- YOUNGBURG GUY. Phosphorus metabolism. III. On the determination of phosphorus in urine, 1145
- AND FABER JASON E. A method for the colorimetric determination of arsenic, 363
- Z
- ZALATEL RALPH P. (See Strickler and Zalatel), 748
- ZIEGLER EDWIN D. The effects on pneumococci of sodium dihydrocholate a bile salt derivative. III, 317

SUBJECT INDEX

Abstracts are indicated by (Abst.) after the page number book reviews by (B Rev) after the page number

A

- Abortus melitensis group, test for agglutination with organisms of 88
- isolation of an organism of 345
- Abstracts 92 201 284 385 495 829 1165 1274
- Acetone tests permanent nitroprussid solution for 375
- Acid response of stomach to test meals of protein fat and carbohydrate 1094
- Acute carbon monoxide poisoning 415
- Adrenal exhaustion experimental 399
- Adrenalin and ephedrine added to local anesthetic solution comparison of the vasoconstrictive action of 773
- Agglutination with organisms of the abortus melitensis group simple presumptive test for 88
- Agranulocytosis 993
 - experimental 829 (Abst.)
 - myeloid cell hyperplasia in bone marrow 1172 (Abst.)
- Allimentary canal of man studies in, 151 240
- Allergic asthma nonspecific desensitization therapy in 495 (Abst.)
- des Lebensalters—die bösartigen Geschwulste 837 (B Rev)
- diseases their diagnosis and treatment 289 (B Rev)
- Allergy of age—the malignant tumors, 837 (B Rev)
- Alveolar air carbon dioxide changes in after subcutaneous histamine injection in human beings 1228
- American physicians and surgeons 838 (B Rev)
- Amyl nitrite effect of upon finger volume 59
- Anaerobic technic, 519
- Anaphylaxis prevention of shock 829 (Abst.)
- Anemia pernicious macrocytosis and erythrocytes and achlorhydria in 829 (Abst.)
 - pigment metabolism and destruction of blood in 823 (Abst.)
 - relation of achlorhydria to 201 (Abst.)
 - treated with massive doses of liver extract 1016
- sickle cell blood picture in 1277 (Abst.)
- phenomenon 913
- Anemias color volume and saturation indexes in value of accurately determined 859
- indexes in color volume and saturation value of accurately determined 859
- secondary aqueous equine liver extract glycerated iron and hemoglobin in treatment of value of 1185
- treatment of 1010
- Aneroid type of tambour for recording respiratory movements and intrathoracic pressure 812
- Anesthesia animal ether bottle for 490
- Anesthetic solution local adrenalin and ephedrine added to comparison of vasoconstrictive action of 773
- Anilin dyes method for spirochetes and moulds with 1169 (Abst.)
- Animals operating table for small 1153
- use of in routine diagnostic work 510
- Antiseptic action of Mallophone 49
- Apparatus for absorbing, expired air of laboratory animals 282
- Arsenic colorimetric determination of 363

- Arthritic patients mono iodo cinchophen treatment of 17
- Arthritis bacteriologic investigations in 1174 (Abst.)
 - deformans, role of streptococcus in, 388 (Abst.)
- Artificial pneumothorax apparatus, recording type of 75
- Aschheim Zondek pregnancy test value and limitations of 61
- test modified for diagnosis of early pregnancy, 354
- Asthma and hay fever in theory and practice, 290 (B Rev)
- Avertin, action of on voluntary and nonvoluntary muscle 1104

B

- B acidophilus medium for 1278 (Abst.)
- B diphtheria stain for 495 (Abst.)
- B mucosus infection of newborn 1171 (Abst.)
- B tuberculosis a potato-egg medium for isolation, 387 (Abst.)
 - demonstration of rare tubercle bacilli in sputum 385 (Abst.)
 - direct culture of from the blood, 830 (Abst.)
- Bacteria in feces pathogenic isolation and identification of 628
- pure smooth and rough colonies at will 830 (Abst.)
- Bacterial mutations 667
- Bacteriologic diagnosis Lyon bile drainage technic as aid to 109
- examination of blood and spinal fluid 566
- Bacteriology and immunity, principles of 106 (B Rev)
 - clinical, 507
 - symposium on 611
 - of bile 583
 - of nose and throat 573
 - of pus 558
 - test tube rack for use in 485
- Bacteriophage III observations on the 681
- factors in preparation of, 285 (Abst.)
- filtrates treatment of colon bacillus infections of urinary tract by 681
- in clinical medicine 675
- Bacterium tularensis method for staining, in tissue sections 193
- Basal metabolism graphs transparent rule for measuring 826
- Bedside interpretation of laboratory findings 105 (B Rev)
- Bendien test for cancer 1170 (Abst.)
- Bile bacteriology of 583
 - determination of cholesterol in 274
 - drainage technic Lyon 109
 - pigment formation effect of liver extract on, 1223
- Bilirubin in blood serum estimation of, 820
- quantitative serum 1
- therapy indications for trial in lobar pneumonia 216
- Bilirubinemia Diazo reaction as a quantitative procedure 1170 (Abst.)
- qualitative and quantitative estimation of 1166 (Abst.)
- Bilirubinuria, methylcane blue test for 385 (Abst.)
- Biochemical and pharmacological study of quinine bi salicylo salicylate, 139

- Biology human and racial welfare, 538 (B Rev)
- Biometrical studies of head lengths of human spermatozoa 297
- Blood and spinal fluid bacteriology of 546 and urine phosphorus normal relationships of 747
- cell counts white in convalescence from infectious diseases 1279 (Abst.)
- cells of origin and maturation of 887
- chlorides determination of using palladium as indicator, 101 (Abst.)
- count diagnostic features of in diseases associated with splenomegaly 1050
- destruction of in pernicious anemia 833 (Abst.)
- determination of quinine in in malaria, as guide in treatment 1172 (Abst.)
- direct culture of B tuberculosis from 830 (Abst.)
- examination technic of 843
- fragility of red corpuscles, 1250
- in infancy and childhood special features of 1043
- lipid variations in the total in lipemia, 1271 (Abst.)
- morphology of diagnostic features of in diseases associated with splenomegaly 1050
- of malarial patients parasites in, enumeration of 1124
- phosphate curves clinical evaluation of 1279 (Abst.)
- physical and chemical studies of human from cases of diabetes mellitus 497
- picture in poiter 1174 (Abst.)
- in hyperthyroidism and in hypothyroidism 1060
- in malignancy 1071
- in sickle cell anemia 1277 (Abst.)
- plasma or serum carbon dioxide changes in after subcutaneous histamine injection in human beings 1228
- pressure tracings manometer for magnification of 482
- reducing power of effects of ultraviolet irradiation on 41
- sedimentation practical value of 1166 (Abst.)
- test in management of pneumothorax patient 1275 (Abst.)
- serum bilirubin in estimation of 820
- sugar following rectal administration of dextrose 834 (Abst.)
- supravital differential counting adapted to clinical use 1263
- transfusion apparatus new Hartman 1266
- by citrate method simple apparatus for 1027
- reactions to 1029
- urea nitrogen determination of by direct nesslerization 1256
- micromethod for 1146
- Blutkrankheiten im lichte der qualitativen blutlehre 393 (B Rev)
- Body fluids, specific gravity of apparatus for quickly measuring 1153
- Bone marrow myeloid cell hyperplasia in 1172 (Abst.)
- structure and function of 951, 960
- tumors of 1179 (B Rev)
- Bones cellularity in various with advancing years of life 960
- Book reviews 103 206 289 393 502 837, 1177
- Brain tumor yellow spinal fluid associated with 496
- Bromsulphalein test in estimation of hepatic function 1274 (Abst.)
- Brucella group, serological determination of smooth strains 1170 (Abst.)
- infections endemic reaction in 833 (Abst.)
- C
- Calcium in saliva determination of 1148
- Calculation of urine solids note on 466
- Cancer Bendaen test for 1170 (Abst.)
- Candiru 103 (B Rev)
- Carbohydrate diet low, in treatment of pre-diabetic state 456
- in treatment of postoperative tetany 754
- Carbon dioxide changes in alveolar air and blood plasma or serum after subcutaneous histamine injection in human beings 1228
- monoxide poisoning, acute and chronic 415
- effect of, on heart 208
- Carcinoma grading the malignancy of 500 (Abst.)
- vs ulcer 1282
- Carotin serum estimation of, 53
- Citric acid and oxygen consumption 14
- Cavernous sinus thrombosis septic 28
- Celloidin sections handling of, device designed to simplify 521
- Cells of the blood origin and maturation of 887
- Cellularity in various bones with advancing years of life variations in 960
- Chemical methods in clinical medicine 1177 (B Rev)
- proof museum tags 832 (Abst.)
- Chemotherapeutics, as aid in diagnosis and treatment of gingivitis 437
- Cholesterol in the bile quantitative determination of 274
- Chronic carbon monoxide poisoning 415
- Chronograph laboratory 185
- Cinchophen molecule action of on liver extract, 17
- Cleaning coverslips apparatus for, 190
- Clinical bacteriology 507
- symposium on 611
- medicine bacteriophage in 675
- chemical methods in 1177 (B Rev)
- intestinal protozoa in, 661
- vaccines in 545
- Conguloflocculation test for malignant tumors 834 (Abst.)
- Cocaine picrotoxin and strychnine detoxification of 225
- Co enzymic activity class experiment on, 1272
- Colloidal gold preparation of 98
- sols, standardizing by utilizing a standard solution of globulin 269
- standardization of in Lange test 259
- solution method for preparation of 1168 (Abst.)
- Colon bacillus infections of urinary tract treatment of by bacteriophage filtrates 681
- Colorimetric determination of arsenic 363
- Coopers modification of Ziehl-Neelson staining method as applied to tubercle bacilli in tissue 1131
- Correspondence 550
- Coverslips, apparatus for cleaning 190
- Creed of a biologist 104 (B Rev)
- Cultural examinations broader aspects of routine 507
- Culture medium from soy bean 1274 (Abst.)
- Cyanhematin standard for the Sahli hemoglobinometer 824
- D
- Dakin's solution simple test for available chlorine strength of 390 (Abst.)
- D Amato leucocytic reaction of, in study of disease 1180
- Decompensated hypertensive heart 211
- Dengue experimental studies in 394
- Denis-Ayer method for estimation of protein in spinal fluid 1175 (Abst.)
- Detoxification of cocaine picrotoxin and strychnine by sodium amygdal 325
- Dextrose rectal administration of blood sugar following 834 (Abst.)
- Diabetes mellitus and the gastric secretion 22
- liver extract in treatment of 1204
- relation of abdominal and rectal infections to the pathogenesis of diabetes mellitus 496 (Abst.)
- study of five hour dextrose tolerance curve in treated diabetic patients 285 (Abst.)

Diagnostic aid in obscure hematologic conditions, 198
 Wolf, use of animals in 510
 Diameter of granulocytes variations in 942
 Drazo reaction as a quantitative procedure in bilirubinemia 1170 (Abst)
 Diet in disease 1178 (B Rev)
 In treatment of prediabetic state 156
 Disease diet in 1178 (B Rev)
 Disinfecting agent merthiolate as 141
 Distilling, column improved 159

E

Eclampsia serum calcium in 1166 (Abst)
 Egg medium simplified for cultivation of tubercle bacilli 1276 (Abst)
 Electrocardiograph in improved form of 804
 Electrodes nonpolarizable 196
 Endermic reaction in brucella infections 800 (Abst)
 Endogenous uric acid and hematopoiesis 428
 Ephedrine and adrenalin added to local anesthetic solution comparison of vasoconstrictive action of 773
 Ergebnisse der medizinischen strahlenforschung 837 (B Rev)
 Erythrocyte size and hemoglobin content of the 899
 Ether bottle for animal anesthesia, improved 490
 Examination for pathogenic fungi 549
 Examinations cultural 507
 Exhaustion experimental adrenal 399
 Experimental adrenal exhaustion 399 studies in dengue 394
 Expired air of laboratory animals apparatus for absorbing substances from 282

I

Fecal protozoa method for staining 493
 Feces pathogenic bacteria in isolation and identification of 628
 Finger volume effect of amyl nitrite upon 59
 Flocculation test a new for serum diagnosis of syphilis 787
 Fluids, body, specific gravity of, apparatus for quickly measuring 1158
 Food allergy 290 (B Rev)
 and protein digestion 503
 Foreign bodies in air passages live fishes impacted in food and air passages of man 499 (Abst)
 in the lung pathologic changes in lung tissue as result of 499 (Abst)
 Fragility of red corpuscles determination in clinical work 1250
 Fungi examination for pathogenic 549
 Fungicides evaluation of 72

G

Gastric peristalsis time relationship of 151
 secretion diabetes and 22
 volume calculation of 240
 Gastrointestinal motility technic contributions to study of 1244
 Gingivitis chemotherapeutics as an aid in the diagnosis and treatment 437
 Glandular fever protozoal nature of the experimental disease 390 (Abst)
 Globulin utilizing a standard solution of a method for standardizing colloidal gold sols 269
 Glucose solution effect of ultraviolet irradiation upon 236
 Colter blood picture in 1174 (Abst)
 Gonococcus complement-fixation test in synovial fluid 39
 Gonorrhea, laboratory aids in diagnosis of in women unreliability of 1275 (Abst)
 the diagnosis of through intracutaneous vaccine injections 497 (Abst)
 Granulocytes diameter of variations in 942

Granulocytopenia, review of, 993
 Gravity of body fluids specific apparatus for quickly measuring 1158

H

Haitian transfusion apparatus new, 1266
 Health examination, significance of periodic, 337
 Heart decompensated hypertensive, 211
 Hematologic conditions diagnostic aid in obscure 198
 Hematology, march of 948
 symposium on, 843, 951
 Hematopoiesis endogenous uric acid and, 428
 Hemoglobin aqueous equine liver extract glycerated iron, and in treatment of secondary anemias, values of 1185
 Hemoglobin content of erythrocyte 899
 Hemoglobinometer Sahli cyanhematin standard for 824
 Hemophilia 932
 mechanism of in infancy and childhood 1278 (Abst)
 Hemopoietic pattern variability of in structure and function of bone marrow 951
 Hepatic function van den Bergh reaction and bromsulphalein test in estimation of 1274 (Abst)
 Histamine injection subcutaneous carbon dioxide changes in alveolar air and blood plasma or serum after 1228
 test in diagnosis of leprosy, 204 (Abst)
 Histopathology of skin diseases 502 (B Rev)
 Honing microtome knives on glass new device, 378
 Huddleson slide test comparison with macroscopic tube test in undulant fever 67
 Human biology and racial welfare 838 (B Rev)
 factor in industry 1177 (B Rev)
 spermatozoa biometrical studies in head lengths 297
 Hydrogen peroxide use of in micro Kjeldahl nitrogen method 272
 Hydrolysis urea in urine determination by 77
 Hyperglycemia relative blood volume in diabetes mellitus 497
 Hypertensive heart decompensated 211
 Hyperthyroidism blood picture in 1060
 Hypothyroidism blood picture in 1060

I

Icterus index 1
 Ideal marriage Its physiology and technic 206 (B Rev)
 Index to chemical action of microorganisms 103 (B Rev)
 Industry human factor in 1177 (B Rev)
 Infection age of leucocyte in relation to 975
 B mucosus in newborn 1171 (Abst)
 in rheumatic state the factor of 1178 (B Rev)
 Infectious diseases staff count in 284 (Abst)
 Inoculation malaria white and differential counts in 985
 Instrument for measuring metabolism a simplified 814
 International congress on asthma 609
 Intestinal protozoa among children in St Louis 133
 in clinical medicine 661
 practical staining method for 1163
 specimens mounted to simulate sigmoidoscopic views 380
 Intradermal test for determination of malignancy 1237
 Intrathoracic pressure aneroid-type of timbour for recording 812

- Iron glycerated aqueous equine liver extract and hemoglobin in treatment of secondary anemias value of 1185
 response of reticulocytes to, 1175 (Abst)
 Isohemagglutinins presence and significance of, in body outside the blood stream 333
 Isolation of streptococci technic for 530

J

- Jaundice relation of to outcome in lobar pneumonia, indications for the trial of bilirubin therapy, 216

K

- Kahn antigen mixer a new for precipitation test 1167 (Abst)
 precipitation tests, microscopic arrangement for reading microscopic, 382
 use of single tube with optimum proportion of serum and antigen 1155
 Klinische laboratoriumstechnik 393 (B Rev)
 Kottman reaction diagnostic value of, in thyroid dysfunction, 195 (Abst)

L

- Laboratory aids in diagnosis of gonorrhea in women unreliability of 1275 (Abst)
 chronograph 438
 diagnosis 1178 (B Rev)
 methods in treatment of pneumonia 594
 procedures mechanical aids in 251
 Lactose in treatment of postoperative tetany 754
 Lange test, method for the standardization of colloidal gold sols in 259
 Lantern slides simple quick and inexpensive method of preparing, 830 (Abst)
 Legal medicine and toxicology 207 (B Rev)
 Leishmania, new solid medium for cultivation of 1278 (Abst)
 Leprosy histamine test in the early diagnosis of 204 (Abst)
 Leucemia proteolytic leucocytic enzyme in 1165 (Abst)
 study and treatment of 1006
 Leucocyte age of in relation to infection, 975
 Leucocytes in convalescence from infectious diseases 1279 (Abst)
 in surgical conditions 1100
 the staff count in infectious disease 284 (Abst)
 Leucocytic reaction of DAmato in the study of disease 1180
 Levinson test in tuberculous meningitis 1166 (Abst)
 Ligating the pylorus in absorption experiments, 369
 Lipemia variations in total blood lipid 1274 (Abst)
 Liver extract aqueous equine glycerated iron and hemoglobin in treatment of secondary anemias value of, 1185
 effect of on bile pigment formation, 1223
 in treatment of diabetes mellitus 1204
 pernicious anemia treated with massive doses of 1016
 tract, action of cinchophen molecule on, 17
 yellow atrophy of acute and chronic with especial reference to its appearance in epidemic form in Sweden in 1927 837 (B Rev)
 Lyon bile drainage technic 109

M

- Macroscopic tube test in undulant fever, 67
 Magnesium cinchophen magnesium oxide (magnophen) preparation treatment of rheumatic fever with, 450
 Malaria, determination of quinine in blood as guide in treatment, 1172 (Abst)
 inoculation white and differential counts in 985
 Malignancy blood picture in, 1071
 in radioactive persons 810
 intradermal test for determination of 1337
 Mallophone relation of the Pu reaction of urine to antiseptic action of 49
 Manometer for magnification of blood pressure tracings 482
 Mechanical aids in laboratory procedures, 251
 Medium for isolation and cultivation of bacteria in the filterable state 286 (Abst)
 Medicine clinical bacteriophage in 675
 intestinal protozoa in 661
 textbook of 289 (B Rev)
 Meningitis, diagnostic value of smears from purpuric lesions in meningococcus bacteremia 387 (Abst)
 new reaction in spinal fluid 1171 (Abst)
 tuberculous Levinson test in 1166 (Abst)
 Meningococcus strains serological and immunological reactions of, 1167 (Abst)
 Mercurochrome—220 soluble use of in treatment of infectious diseases of the skin 496 (Abst)
 Merthiolate as a skin disinfecting agent 443
 Metabolism graphs basal transparent rule for measuring 326
 phosphorus, 1145
 pigment and destruction of blood in pernicious anemia, 833 (Abst)
 simplified instrument for measuring 814
 Micro Kjeldahl method an improved 79
 nitrogen method use of hydrogen peroxide in 372
 Micromethod for blood urea nitrogen 1146
 Microorganisms respiration of 731
 Microscope use of 104 (B Rev)
 Microscopic arrangement for reading macroscopic Kahn precipitation tests 382
 Microtome knives honing on glass 378
 knife sharpener 253
 Milk protein in analysis of, 373
 Mono-iodo cinchophen treatment of arthritic patients 17
 Mononucleosis infectious presence of heterophile antibodies in, 332 (Abst)
 Multiple sclerosis study of etiology of 392 (Abst)
 Muscle action of avertin on 1104
 Museum specimens, counting of 1279 (Abst)
 tags chemical proof, 832 (Abst)
 Myeloid cell hyperplasia in bone marrow 1172 (Abst)

N

- Nephritic blood, nonglucose reduction present in normal and increased 471
 Nephritis treatment of 292
 Neurosyphills malarial therapy of other than uncomplicated dementia paralytica, 386 (Abst)
 Neutrophilic graph 962
 Newborn, B mucosus infection in 1171 (Abst)
 Nitroprussid solutions for acetone test 375
 Nonelastic bulb for pipettes 280

Nonglucose reduction present in normal and increased in nephritic blood 171
 Nonpolarizable electrodes 196
 Nose and throat bacteriology of, 573

O

Operating table for small animals 1153
 Ova method for examining urine for Helminth eggs 94 (Abst)
 Oxygen consumption catamenia and 11
 Ozena bacteriology bacillus ozeni foetidie perex and bacillus proteus in 500 (Abst)

P

Papain preparations intended for surgical use sterilization and standardization of 459
 Paraffin embedding warm plate for 251
 technic rapid 1162
 Parasites in blood of malarial patients enumeration of 1124
 Pathogenic bacteria in feces isolation and identification of 628
 Pathology textbook of 106 (B Rev)
 Periodic health examination significance of 337
 Pernicious anemia treated with massive doses of liver extract 1016
 Pertussis isolation and cultivation of H pertussis 286 (Abst)
 Petri dish holder new for counting and fishing colonies 85
 Pu reaction of urine relation of the to antiseptic action of mallophone in vitro 49
 values of urine determination of 1133
 Pharmacologic study of quinine bisalicylosalicylate 139
 Phosphates in sugar tolerance 1277 (Abst)
 Phosphorus in saliva determination of 1148
 in urine determination of 1145
 metabolism 1145
 normal relationships of blood and urine 347
 Photography surgical a new and simplified technic 81
 x-ray ink 391 (Abst)
 Photometer the Vernes-Bricq-Yvon 792
 Photomicrographic camera 255
 Physicians and surgeons American 838 (B Rev)
 Physiology correlations and technic of the van den Bergh reaction icterus index and quantitative serum bilirubin 1
 Pigment metabolism and destruction of blood in pernicious anemia 833 (Abst)
 Pipettes nonelastic bulb for 280
 Pneumococci effects of sodium dehydrocholate on 317
 immediate typing of 331 (Abst)
 rapid method of typing 92 (Abst)
 Pneumococcus typing a microscopic method by the use of stained organisms 201 (Abst)
 Pneumonia immune transfusion in lobar 386 (Abst)
 laboratory methods in treatment of 594
 lobar relation of jaundice to 216
 specific therapy of 107
 Pneumothorax apparatus artificial recording type 75
 patient, blood sedimentation test in management of 1275 (Abst)
 Polymycolitis 401
 results of treatment in one hundred and four cases 391 (Abst)
 spinal fluid cytology 388 (Abst)
 Polynuclear count as advocated by Schilling 169

Postoperative tetany, carbohydrate in treatment of 751
 Potentials, glass electrode simple vacuum-tube potentiometer for measurement of 1268
 Potentiometer vacuum-tube, a simple for measurement of glass electrode potentials 1268
 Practical physiological chemistry, 105 (B Rev)
 Prediabetic state, 456
 weight reduction in 456
 Precipitation test a new Kahn antigen mixer 1167 (Abst)
 Pregnancy rapid method of diagnosis 100 (Abst)
 serum diagnosis of 96 (Abst)
 Preparation of vaccine 538
 Pressure system for formalin alcohol, and distilled water, 252
 Protein digestion and food allergy, 503
 in milk analysis of 373
 in spinal fluid Denis-Ayer method for estimation of, 1175 (Abst)
 Protozoa examination and identification of, 639
 fecal method for staining 493
 intestinal among children of St Louis, 133
 in clinical medicine 661
 practical staining method for 1163
 Pulmonary tuberculosis sputum examination in 611
 Pus bacteriology of, 558
 Pylorus ligating the in absorption experiments 369

Q

Quinine bisalicylo salicylate biochemical and pharmacologic study of, 139
 in blood determination of in malaria as guide to treatment 1172 (Abst)

R

Radiation medical results of the investigations of, 837 (B Rev)
 Radioactive persons malignancy in 840
 Reactions to blood transfusion 1029
 Red corpuscles fragility of determination in clinical work 1250
 Regulation of size as illustrated in unicellular organisms 502 (B Rev)
 Regulierung der Atmung 393 (B Rev)
 Rehfuß tube competency of as a complete evacuator 1120
 Resistance to infectious diseases 105 (B Rev)
 Respiration of microorganisms 731
 Respiratory movements aneroid-type of tambour for recording 812
 Reticulocytes response of to iron 1175 (Abst)
 staining of 1174 (Abst)
 Rheumatic fever treatment of with magnesium cinchophen magnesium oxide (magnphen) preparation 450
 state factor of infection in 1178 (B Rev)
 Rheumatism recent advances in the study of 291 (B Rev)
 Ringworm asymptomatic frequency of as occurring in the more common cutaneous affections 743
 Rube virulence test and sedimentation test comparison of in gynecologic cases 1276 (Abst)

S

Sahlb hemoglobinometer, cyanhematin standard for the 824
 Sahlb calcium and phosphorus in determination of 1148

- Schilling polynuclear count 169
 Sections colloidal handling of device designed to simplify 821
 Sedimentation blood practical value of 1166 (Abst)
 test and Ruge virulence test comparison of in gynecologic cases 1276 (Abst)
 Septic cavernous sinus thrombosis 28
 Serology of syphilis, studies in the, VII, 778
 VIII 787
 test tube rack for use in 185
 Serum and antigen optimum portion use of single tube with for Kahn precipitation test 1155
 calcium in eclampsia, 1166 (Abst)
 carotin estimation of 53
 diagnosis of syphilis new flocculation test for, 787
 Sickie cell anemia, blood picture in 1277 (Abst)
 phenomenon 313
 Sickling in moist preparations rate of 913
 Sinus thrombosis septic cavernous 28
 Skin disinfecting agent merthiolate as 443
 Sodium dehydrocholate a bile salt derivative 317
 Soy bean culture medium from 1274 (Abst)
 Specific therapy of pneumonia 107
 Spermatozoan human biometrical studies of head lengths 297
 Spinal fluid a new test 390 (Abst)
 bacteriologic examination of 566
 Denis-Ayer method for estimation of protein in 1175 (Abst)
 new reaction in in meningitis, 1171 (Abst)
 Splenomegaly diseases associated with diagnostic features of blood count and morphology of blood in 1050
 Split second timer 376
 Sputum examination in pulmonary tuberculosis 611
 tubercle bacilli in rapid staining of 1275 (Abst)
 Stain differential stain for diagnosis of Neisserian infection 95 (Abst)
 for non-acid fast tubercle bacilli, 832 (Abst)
 method for spirochetes and moulds with anilin dyes 1169 (Abst)
 Wrights modification of use of 818
 Staining bacterium tularensis in tissue sections 193
 of reticulocytes 1174 (Abst)
 supravital clinical applications of 921
 urinary sediment new method for 1168 (Abst)
 Sterilization and standardization of papain preparations intended for surgical use 459
 Stomach, acid response of to test meals of protein fat and carbohydrate 1094
 Streptococci technic for isolation of 530
 Sugar tolerance curves clinical evaluation of 1279 (Abst)
 phosphates in 1277 (Abst)
 Supravital differential counting adapted to clinical use 1263
 staining clinical applications of 921
 Surgical conditions leucocytes in 1100
 photography new and simplified technic 81
 Symposium on clinical bacteriology 507
 608 611
 on hematology 843 951
 Synovial fluid gonococcus complement-fixation test in 39
 Syphilis flocculation test for serum diagnosis of a new 787
 serology of studies in the VII 778
 VIII 787
 serum diagnosis of new flocculation test for 787
- T
 Tambour, incoid-type of for recording respiratory movements and intrathoracic pressure 812
 Technic, anaerobic 519
 Test meals acid response of stomach to 1094
 tube rack for use in serology and bacteriology 185
 Tertiary postoperative carbohydrate in treatment of 754
 Thermocouple and thermopile for determination of temperature in biology and medicine 181
 Thermopile and thermocouple for determination of temperature in biology and medicine, 181
 Throat bacteriology of 573
 Tissue, a new clearing and mounting fluid for small insects 203 (Abst)
 method for examination of the appendix 389 (Abst)
 modification of Mallory-Heidenhain's differential staining method and adaptation to formalin-fixed material 202 (Abst)
 paraffin technic rapid 1162
 preparation and staining large bone sections 96 (Abst)
 rapid and permanent stain for myelin sheaths 100 (Abst)
 stain modification of Mallory's triple stain 93 (Abst)
 Trauma disease compensation 206 (B Rev)
 Transfusion apparatus blood new Hartman 1266
 of blood by citrate method simple apparatus for 1027
 Treatment of nephritis 292
 of pneumonia laboratory methods in 594
 of rheumatic fever with magnesium cinchophen magnesium oxide (magphen) preparation 450
 Tryptophanuria clinical incidence of, 65
 Tubercle bacilli in blood stream of rabbits during course of infection 1176 (Abst)
 in tissue Coopers modification of Ziehl-Neelson staining method as applied to 1131
 rapid staining of in sputum 1276 (Abst)
 simplified egg medium for cultivation of 1276 (Abst)
 stain for non-acid-fast bacilli and granules 832 (Abst)
 Tuberculous meningitis Levinson test in 1166 (Abst)
 Tuberculosis and aspergillosis association 498
 direct culture in tuberculous effusions 386 (Abst)
 of B tuberculosis from the blood 330 (Abst)
 parenteral BCG vaccination 1169 (Abst)
 pulmonary sputum examination in 611
 Tumors coaguloflocculation test for malignant 834 (Abst)
 malignant coaguloflocculation test for 834 (Abst)
 of bone 1179 (B Rev)
- U
 Ueber die akute und chronische gelbe Leberatrophie mit besonderer Berücksichtigung ihres epidemischen Auftretens in Schweden im Jahre 1927 837 (B Rev)
 Ulcer vs carcinoma 1282
 Ultraviolet irradiation effect of on glucose solution 236
 on reducing power of the blood 44
 Undulant fever tests 67

Unicellular organisms regulation of size as illustrated in 502 (B Rev)
 Urea in urine determination of by means of hydrolysis 77
 nitrogen blood determination of by direct nesslerization 1256
 micromethod for 1116
 Uric acid, endogenous and hematopoiesis 428
 Urinary sediment staining new method for 1168 (Abst)
 tract colon bacillus infections of, treatment of by bacteriophage filtrates 681
 Urine number of formed elements in heart disease 203 (Abst)
 Pu values of determination of 1133
 phosphorus in determination of 1145
 solids note on calculation of 466

V

Vaccination parenteral BCG and tuberculosis 1163 (Abst)
 Vaccine therapy principles of 202 (Abst)
 Vaccines in clinical medicine 545
 preparation of 538
 Vacuum-tube potentiometer simple for measurement of glass electrode potentials 1268
 Van den Bergh reaction in estimation of hepatic function 1274 (Abst)
 technic of 1

Vasoconstrictive action of adrenalin and ephedrine added to local anesthetic solution, comparison of 773
 Vernes-Bréq-ivon photometer 792
 Visceral disease, symptoms of, 839 (B Rev)

W

Wassermann positive supposed artificial induction of in originally negative human sera 778
 reaction rational use of 498 (Abst)
 Weight reduction in treatment of prediabetic state 456
 Wrights stain modification of use of 818

X

X-ray technology 104 (B Rev)

Y

Yellow atrophy of liver acute and chronic with especial reference to its appearance in epidemic form in Sweden in 1927 837 (B Rev)

Z

Ziehl-Neelson staining method Coopers modification of as applied to tubercle bacilli in tissue 1131

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